UNIVERSITATEA DE ŞTIINŢELE VIEŢII
„REGELE MIHAI I" DIN TIMIŞOARA

FACULTATEA DE MEDICINĂ VETERINARĂ

LUCRĂRI ŞTIINŢIFICE

MEDICINĂ VETERINARĂ
TIMIŞOARA
VOLUMUL LVI (1)

SCIENTIFICAL PAPERS
VETERINARY MEDICINE

To be cited: LUCRARI STIINTIFICE: MEDICINA VETERINARA TIMISOARA (SCIENTIFICAL PAPERS: VETERINARY MEDICINE TIMISOARA)**,** vol. LVI (1), 2023

Available online at: **https://www.usab-tm.ro/ro/publicatii-42/revista-47-volume-de-lucrari-stiintifice-10682**
Indexed and/or abstracted in: **CABI Full Text, CAB Abstracts, Ulrich's Periodicals Directory**
Editor: AGROPRINT TIMISOARA ISSN: 2668-2435 and ISSN-L 1221-5295
Printed by: IMPRIMERIA MIRTON TIMISOARA

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TESTING THE EFFICACY OF IVERMECTIN 2% (ORAL PASTE) BY FECRT METHOD IN HORSES FROM TIMIȘ COUNTY

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Summary

The development of chemoresistance (an inherited trait of larvae from adult parasites) in the cyathostomin population to one or more antiparasitic substances is a highly topical issue. A very good method for identifying chemoresistance is the faecal egg count reduction test (FECRT), a cheap but effective method, which is also considered the gold-standard. This method is based on the results (number of eggs) obtained before (day 0) and after treatment (day 14), in the same horses. The objective of this study was to establish the efficacy of ivermectin 2% in horses from several cities from Timiș County. Fresh faecal samples were taken from 75 horses (five cities) from Timiș County, only horses that had several eggs per gram of faeces (EPG) greater than or equal to 200 were selected. Larval cultures were also taken to identify cyathostomins and large strongyles. 55 horses were tested for the effectiveness of ivermectin 2% and 20 horses had EPG<200 and were excluded from testing. Before the treatment larvae of *Strongylus vulgaris* (23.63%) in 13 horses and larvae of the subfamily *Cyathostominae (*100%) in all 55 horses, with different percentages for types A, B, C, D, F and *Gyalocephalus capitatus* were identified. After treatment only cyathostomins larvae type A and D were identified in 1 group of horses. In 55 horses administered ivermectin 2% (oral paste, 20mg/100 kg body weight, single dose) it was observed that both individually and in groups, the antiparasitic substance showed efficacy (>95%).

Keywords: cyathostomins, chemoresistance, ivermectin, horses.

Due to the resistance of the IBD virus in the environment, to the action of usual disinfectants, as well as its persistence in the body of insects, general prophylaxis measures

Cyathostominosis normally evolves without specific clinical signs, except for larval cyathostominosis, and for this reason tests must be carried out to assess the efficacy of antiparasitic substances, as chemoresistance is otherwise difficult to recognize (19). Chemoresistance evolves slowly over several years and may develop without clinical signs, until late stages when therapeutic efficacy fails, and clinical signs appear (17).

Causes of chemoresistance may be under-dosing of the antiparasitic substance (frequently the body weight of the horse is approximated), frequent deworming, use of the same antiparasitic substance for several consecutive years, even the whole life of the animal, etc. Larval stages in the intestinal mucosa (hypobiotic), pose a health hazard to equines, being a reservoir for the transfer of chemoresistant parasites to new locations with the movement/ displacement of equines; antiparasitic treatment should be carried out considering the hypobiotic

larval stages of cyathostomins - being considered an important principle of parasitological control (11, 12, 21). Another cause may be the easy access of equine owners to purchase antiparasitic substances, as they do not consider the phenomenon of chemoresistance and usually administer the economically convenient product.

The objective of this study was to establish the efficacy of ivermectin 2% (oral paste, 20mg/100 kg body weight, single dose) in horses from several cities from Timiș County.

A very good method is the faecal egg count reduction test (FECRT). It can be used for all classes of antiparasitic drugs, is relatively simple to perform, and is currently the most used method, being called the gold-standard for identifying chemoresistance (13, 16, 19, 20).

Materials and methods

The study was carried out between October 2013 and March 2017, on 75 horses (5 groups), of Timiș County. The horses taken in the study were part of both private herds and private households, belonging to hot blood, warm blood, cold blood horses. An important criterion for the selection of the horses was that the animals had not been dewormed for at least 12 weeks prior to the start of the study. Fresh faecal samples were collected and packed in suitably vacuumed and labelled plastic bags, then kept in a cool box until arrival at the Parasitology and Parasitic Diseases Clinic of the Faculty of Veterinary Medicine, where coproparasitological examinations were performed.

The Willis (a flotation method for the identification of light parasite eggs) and McMaster (a flotation method to determine the degree of infestation of horses with parasites, EPG - eggs per gram of faeces) methods were performed (4, 6, 8). For the treatment and evaluation of the efficacy of the antiparasitic substances, horses with EPG≥200 were selected.

The FECRT was conducted according to the guidelines of the" World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance". Larval cultures following Roberts and O'Sullivan's modified technique were also performed (15, 14). To identify cyathostomins and large strongyles larvae (L3) were used determination keys by Madeira de Carvalho (9, 10).

$$FECRT\ \% = \frac{\text{EPG pre-treatment (0 day)} - EPG\ \ 14\ day\ post-treatment}{\text{EPG}\ \ 0\ \text{day}}\ x\ 100$$

An efficacy below 95% for macrocyclic lactones (ivermectin) indicates that resistance is suspected (5, 7).

Results and discussions

Number of 20 horses were excluded from the study due to low egg count. The 55 horses were dewormed with ivermectin oral paste, 20mg/ 100 kg body weight, single dose (Table 1).

On the day of treatment, cyathostomins larvae were identified in all samples from which larval cultures were taken (55 faecal samples), Strongylus vulgaris (23.63% larvae in 13 faecal samples out of 55) (Table 2).

Table 1

The examined horses, dewormed with ivermectin 2% and not dewormed

City/ group	Breed	Farm horses/private households	Total number of horses	Excluded horses- not dewormed	Horses dewormed		
					EPG≥200	Males	Females
TM 1	HBH	Farm horses	8	4	4	1	3
TM 2	WBH, HBH	Private households	16	4	12	4	8
TM 3	HBH	Farm horses	6	3	3	2	1
TM 4	HBH, CBH	Farm horses	37	5	32	10	22
TM 5	HBH	Private households	8	4	4	2	2
Total			**75**	**20**	**55**	**19**	**36**

Note: HBH=hot blood horses; WBH=warm blood horses; CBH=cold blood horses

Table 2

L3 found in larval cultures from 55 horses

County	City/ group	Pre-treatment, larval cultures, 0 Day		Post-treatment, larval cultures, 14 Day	
		L3 of subfamily *Cyathostominae*	L3 of subfamily *Strongylinae*	L3 of subfamily *Cyathostominae*	L3 of subfamily *Strongylinae*
Timiș	TM 1	Type A, D, F, *Gyalocephalus*	-	-	-
	TM 2	Type A., B, D	*Strongylus vulgaris*	-	-
	TM 3	Type A, C, D	-	-	-
	TM 4	Type A, C, D, F	-	Type A, D	-
	TM 5	Type A, D, F	*Strongylus vulgaris*	-	-

EPG in pre-treatment day was between 200 and 3850 for the 5 groups (Table 3).

The highest EPG values were found in horses in TM 4. The EPG values recorded on 0 day and on 14 day are shown in the next table together with the average and standard deviations EPG values for the different groups (Table 3).

Table 3

EPG values found in examined horses from the study

City/group	EPG – 0 Day A.M./Lim.	SD	EPG – 14 Day A.M./Lim.	SD
TM 1	1062.5/ 250-2500	990.26	0	0
TM 2	233.33/ 200-350	44.38	0	0
TM 3	216.66/ 200-250	28.86	0	0
TM 4	1429.68/ 200-3850	1138.67	14.06/ 0-150	31.71
TM 5	375/ 200-600	206.15	0	0

Note: A.M. = arithmetic mean; Lim. = limits; SD = standard deviation.

The groups of horses, which were administered ivermectin 2% had a FECRT between 99.01-100% and individually, the horses in TM 1, TM 2, TM 3, TM 5 had a FECRT of 100%, and TM 4 (96-100%) (Table 4, 5).

Table 4

FECRT values for Ivermectin 2%, found in individual samples on the 5 groups

.

City/Group	FECRT-limits	Number of horses	Number of horses - efficacy (>95%)	Horse number - suspected chemoresistance (<95%)
TM 1	100%	4	4	-
TM 2	100%	12	12	-
TM 3	100%	3	3	-
TM 4	96-100%	32	32	-
TM 5	100%	4	4	-

Smith et al. in 2015 conducted a study in the United States in which 98.84% efficacy for ivermectin and the presence of resistance for fenbendazole (6.15%) was recorded (18).

Cernea et al. in 2015, conducted a study on the chemoresistance of the strongylid population to fenbendazole and ivermectin in Romania, identifying the presence of the cyathostomine population (types A, B, C, D, E, F, G, H,

Gyalocephalus, Poteriostomum), but also those of the subfamily Strongylinae. Chemoresistance has not been encountered with ivermectin, preserving its efficacy (3).

Table 5

The efficacy of ivermectin 2% on animals' group

City/Group	Number of horses	FECRT
TM 1	4	100%
TM 2	12	100%
TM 3	3	100%
TM 4	32	99.01%
TM 5	4	100%

Buzatu et al. (2) in 2015 conducted a study from March 2013 to September 2014. Three Romanian stallion farms were tested for the efficacy of ivermectin (108 mares) and fenbendazole (29 mares). They demonstrated the efficacy of ivermectin (98.2-100%), but for fenbendazole chemoresistance was suspected (75.4%) (2).

In 2021, Butler et al. (1) used the FECRT to determine the presence of anthelmintic resistance (AR), while egg reappearance periods (ERP) are used to investigate early macrocyclic lactone resistance. Faecal egg count reduction tests performed after ivermectin administration determined the lack of cyathostomins chemoresistance in all farms studied (1).

Conclusions

On the day of treatment, cyathostomins larvae were identified in all samples from which larval cultures were taken (55 faecal samples), Strongylus vulgaris (23,63% larvae in 13 faecal samples out of 55.

EPG in pre-treatment day was between 200 and 3850 for the 5 groups. The highest EPG values were found in horses in TM 4.

The horse groups administered ivermectin 2% had an FECRT between 99.01-100%, indicating that the antiparasitic substance had a high efficacy against cyathostomins, but also against strongyles of the Strongylinae subfamily.

Individually, horses administered 2% ivermectin showed high efficacy of 96-100%.

References

1. **Butler, A.J., Greenbank, H., Parrish, R., Nielsen, M.K., Stoughton W.B.,** Prevalence of anthelmintic resistant cyathostomins in Prince Edward Island, Canada, Veterinary Parasitology, 2021, 26, 100629.

2. **Buzatu, M.C., Mitrea, I.L., Miron, L., Ionita, M.,** Efficacy of two anthelmintic products on strongyles in horses from stud farms in Romania, Agriculture and Agricultural Science Procedia, 2015, 6, 293-298.
3. **Cernea, M., Cristina, R.T., Ștefănuț, L.C., Madeira de Carvalho, L.M., Taulescu, M.A., Cozma, V.,** Screening for anthelmintic resistance in equid strongyles (Nematoda) in Romania, Folia Parasitologica, 2015, 62, 023.
4. **Coles, G.C., Bauer, C., Borgsteede, F.H., Geerts, S., Klei, T.R., Taylor, M.A., Waller, P.J.,** World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance, Veterinary Parasitology, 1992, 44, 35-44.
5. **Coles, G.C., Jackson, F., Pomroy, W.E., Prichard, R.K., von Samson-Himmelstjerna, G., Silvestre, A., Taylor, M.A., Vercruysse, J.,** Review The detection of anthelmintic resistance in nematodes of veterinary importance, Veterinary Parasitology, 2006, 136, 167-185.
6. **Cosoroabă, I., Dărăbuş, G., Oprescu, I., Morariu, S.,** Diagnostic paraclinic şi tehnici experimentale în parazitologie, 1st ed., Ed. Mirton, Timişoara, 2002.
7. **Craven, J., Bjørn, H., Barnes, E.H., Henriksen, S.A., Nansen, P.,** A comparison of in vitro tests and a faecal egg count reduction test in detection of anthelmintic resistance in horse strongyles, Veterinary Parasitology, 1999, 85, 49-59.
8. **Lester, H.E., Matthews, J.B.,** Faecal worm egg count analysis for targeting anthelmintic treatment in horses: points to consider, Equine Veterinary Journal, 2014**,** 46, 139-145.
9. **Madeira de Carvalho, L.M.,** Horse strongyles L3 larval stages. Morphological characters relevant for their identification, 17th Conference WAAVP, Workshop Systematics of the *Cyathostominae* of horses, Copenhagen, Denmark, 1999, 6.
10. **Madeira de Carvalho, L.M.,** Morphology of eggs and third stage infective larvae (L3) of intestinal strongyles (Nematoda: Strongylidae) in domestic equids. Culture, collection, and identification methods of infective larvae. (In portuguese), Report of a practical class for the Proves of Pedagogical Ability and Scientific Capability (equivalent to MSc.), Faculty of Veterinary Medicine, Technical University of Lisbon, 1991, 8.
11. **Mason, M.E., Voris, N.D., Ortis, H.A., Geeding, A.A., Kaplan, R.M.,** Comparison of a single dose of moxidectin and a five-day course of fenbendazole to reduce and suppress cyathostomin fecal egg counts in a herd of embryo transfer-recipient mares, Journal of the American Veterinary Medical Association, 2014, 245, 8, 944-951.
12. **Matthews, J.B.,** An update on cyathostomins: anthelmintic resistance and worm control, Equine Veterinary Journal, 2008, 20, 552-560.
13. **Peregrine, A.S., Molento, M.B., Kaplan, R.M., Nielsen, M.K.,** Review Anthelmintic resistance in important parasites of horses: Does it really matter, Veterinary Parasitology, 2014, 201, 1-8.
14. **Reinecke, H.K.,** The larval anthelmintic test in ruminants, Technical

communication, Department of Agricultural Technical Services, South Africa, 1973, 106, 20.
15. **Roberts, F.H.S., O'Sullivan, P.J.,** Methods for egg counts and larval cultures for strongyles infesting the gastro-intestinal tract of cattle, Australian Journal of Agricultural Research, 1950, 1, 1 99-102.
16. **Rose Vineer, H., Vande Velde, F., Bull, K., Claerebout, E., Morgan, E.R.,** Attitudes towards worm egg counts and targeted selective treatment against equine cyathostomins, Preventive Veterinary Medicine, 2017, 144, 66-74.
17. **Sangster, M.C.,** Pharmacology of anthelmintic resistance in cyathostomes: will it occur with the avermectins/milbemycins?, Veterinary Parasitology, 1999, 85, 189-204.
18. **Smith, M.A., Nolan, T.J., Rieger, R., Aceto, H., Levine, D.G., Nolen-Walston, R.,** Efficacy of major anthelmintics for reduction of fecal shedding of strongyle – type eggs in horses in the Mid-Atlantic region of the United States, Veterinary Parasitology, 2015, 214, 139-143.
19. **Vidyashankar, A.N., Hanlon, B.M., Kaplan, R.M.,** Statistical and biological considerations in evaluating drug efficacy in equine strongyle parasites using fecal egg count data, Veterinary Parasitology, 2012, 185, 45-56.
20. **Von Samson-Himmelstjerna, G.,** Anthelmintic resistance in equine parasites-detection, potential clinical relevance and implications for control, Veterinary Parasitology, 2012, 185, 2-8.
21. *** American Association of Equine Practitioners. AAEP parasite control guidelines, www.aaep.org/custdocs/ParasiteControlGuidelinesFinal.pdf

THERAPEUTIC INDICATIONS AND TECHNICAL FEATURES IN PERITONEAL DIALYSIS IN CATS

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Summary

The most important indication for peritoneal dialysis in dogs and cats is anuria caused by acute kidney injury (AKI) refractory to fluid therapy. Dialysis is also indicated in patients with severe acute non-anuric uremia, in whom the blood urea nitrogen (BUN) exceeds 100 mg/dl or in whom the creatinine level is greater than 10 mg/dL. Peritoneal dialysis is also indicated in case of increased levels of creatinine and urea, hyperkalemia, hyperphosphatemia, hypercalcemia or metabolic acidosis, when they do not yield to other treatments. Peritoneal dialysis is contraindicated in patients with peritoneal adhesions, fibrosis, malignant tumors and pleuroperitonitis. The presence of adhesions and fibrosis reduces the surface area and efficiency of peritoneal dialysis. Patients with a severe hypercatabolic status may experience complications due to massive protein loss correlated with the duration of peritoneal dialysis.
The 2 types of peritoneal dialysis practiced in cats are continuous peritoneal dialysis and intermittent peritoneal dialysis. The ideal catheter for peritoneal dialysis allows the adequate administration and evacuation of the dialysate, causes minimal subcutaneous losses, minimizes infection, both in the peritoneal cavity and in the subcutaneous tissue. The specific composition of the dialysate is an important factor to consider when performing peritoneal dialysis. Dialysate solutions differ based on the buffer component, electrolytes and/or osmotic agents used. The ideal peritoneal dialysis solution should achieve low-absorption clearance of osmotic agents, supply deficient electrolytes and nutrients, correct acid-base problems, inhibit microbial growth, and be inert to the peritoneum.
Keywords: indications, technics, peritoneal dialysis, cats.

Indications for peritoneal dialysis

The first and foremost indication for peritoneal dialysis in dogs and cats is anuria from acute kidney injury (AKI) refractory to fluid therapy. Dialysis is also indicated in patients with severe acute nonanuric uremia, in whom the blood urea nitrogen (BUN) exceeds 100 mg/dl or in whom the creatinine level is greater than 10 mg/dl (7).

Peritoneal dialysis should be considered in a uremic patient when attempts to establish urine flow have failed. Peritoneal dialysis may also be used to stabilize patients with uroabdominal or other urinary tract obstructions prior to surgery to help overcome anesthetic shock (Fig.1) (14).

In cats, peritoneal dialysis is also indicated in conditions that cause an increase in the amount of toxins in the blood and plasma. Among these diseases, the most common in cats are: acute kidney damage or chronic kidney disease, ethylene glycol or barbiturate poisoning, pulmonary edema, uremia, hyperhydration

states, in the latter case hypertonic dialysate is used (5).

Peritoneal dialysis is also indicated in case of increased levels of creatinine and urea, hyperkalemia, hyperphosphatemia, hypercalcemia or metabolic acidosis, when they do not yield to other treatments (10).

Peritoneal dialysis can be used successfully in various intoxications and metabolic imbalances. It can remove dialyzable toxins such as ethylene glycol, ethanol, barbiturates, propoxyphene, hydantoin and correct electrolyte imbalances such as hyperkalemia (2).

BUN (mg/dl) = Urea (mg/dl) / 2.1428

Urea (mmol/l) = BUN (mg/dl) * 0.357

BUN: 1 mg/dL = 2.80112 mmol/L

Urea: 1 mg/dL = 0.00166 mmol/L

Creatinine: 1 mg/dL = 0.01131222 µmol/L

Fig. 1. Conversion of renal constants by measure unit and interpretation (17)

In the case of each animal that receives the indication for peritoneal dialysis, a rigorous clinical examination is performed, supplemented by biochemical and hematological analyses, as well as urine examination. It is important to know the evolution of the condition, as well as the existence of other conditions or the medication used in the case of each patient (11).

However, peritoneal dialysis is limited in its ability to remove toxins from the blood and is about one-eighth to one-fourth as efficient as hemodialysis. Peritoneal dialysis is indicated in situations where hemodialysis, hemofiltration, or hemoperfusion with charcoal particles are not available, vascular access is difficult to achieve, or the patient has refractory hypotension, making hemodialysis a high-risk procedure (1).

When peritoneal dialysis is performed for poisonings that have life-threatening side effects (hyperkalemia, ethylene glycol poisoning), very frequent exchanges should be performed to achieve a faster rate of clearance of electrolyte abnormalities such as hyperkalemia and hypercalcemia. The last two can also be effectively managed by hemodialysis (8).

Conditions that cause life-threatening hypothermia can also be corrected with peritoneal dialysis. The concentration of electrolytes in the dialysate solution should be similar to that in plasma to prevent extreme or rapid electrolyte fluctuations (18).

In the case of hypothermia that endangers the patient's life, it is instilled in

the abdomen, dialyzed at a temperature of 42ºC-43ºC, with a body rewarming rate of 1ºC-2ºC per hour. In the case of hyperthermia that endangers the patient's life, the dialysate should be administered at room temperature (12).

Peritoneal dialysis has also been used extensively in human neonates with urea cycle disorders. This is used as an emergency tool to correct hyperammonemia along with additional medical management to stabilize this condition until liver transplantation. It has recently been shown that detoxification by peritoneal dialysis in this case is more effective than by hemodialysis or continuous hemofiltration. (4)

There are no studies in veterinary patients to evaluate the utility of peritoneal dialysis in the treatment of life-threatening hyperammonemia secondary to hepatic encephalopathy or urea cycle disorders (16).

Congestive heart failure refractory to medical management is another indication in human medicine for peritoneal dialysis. A recent study looked at patients with severe congestive heart failure and found decreased mortality with the use of hemofiltration followed by peritoneal dialysis compared to patients with similar heart conditions (17).

This concept can also be applied to patients with severe overload. Exchanges should be performed every hour in severe fluid overload, and a significantly hyperosmotic dialysate (4.25%) should be used to encourage ultrafiltration (6).

Contraindications for peritoneal dialysis

Peritoneal dialysis is contraindicated in patients with peritoneal adhesions, fibrosis, malignant tumors and pleuroperitonitis. The presence of adhesions and fibrosis reduces the surface area and efficiency of peritoneal dialysis. Patients with a severe hypercatabolic status may experience complications due to the massive protein loss associated with the duration of peritoneal dialysis (9).

Relative contraindications are imposed in patients with recent abdominal surgery and inguinal or abdominal hernias due to the risk of increased intraperitoneal pressure. Patients in a severe hypercatabolic state, such as fire victims or severely malnourished patients, have a relative contraindication due to their tendency to lose protein through the peritoneum during dialysis. Patients with recent abdominal surgery, especially those with gastrointestinal procedures, are at risk for dehiscence and infection during peritoneal dialysis because of increased intraperitoneal pressure and the potential for extravasation of dialysis fluid through the incision site (15).

Peritoneal dialysis also carries an increased risk of complications in patients with polycystic kidney disease, extreme obesity, peripheral vascular disease, and hyperlipidemia (19).

Types of peritoneal dialysis

The 2 types of peritoneal dialysis practiced in cats are continuous peritoneal

dialysis and intermittent peritoneal dialysis (1).

Continuous peritoneal dialysis is based on the use of 2 cannulas that allow a continuous flow of the dialysate in the peritoneal cavity. It is based on the principle of gravity, so a bag with dialysate (heated to body temperature) is positioned at a height, which allows the blood to be purified of toxins and excess water, and an empty bag is positioned at a lower level, which allows the capture of the liquid that is externalized from the body. This exchange lasts 45 minutes and can be done once a day, as it is necessary to perform this type of therapy daily (20).

Intermittent peritoneal dialysis has as a procedure the introduction of a volume of liquid and its extraction after a certain period by means of the same catheter. The volume of dialysate introduced varies depending on the concentration, composition and individual needs of the patient, being on average 40-60 ml/kg/day (13).

Types of peritoneal catheters

Peritoneal dialysis is a simple process that requires a minimum of equipment, so its success depends on the type of catheter used and its location. (5).

The ideal catheter for peritoneal dialysis allows the administration and adequate evacuation of the dialysate, determines minimal subcutaneous losses, minimizes infection, both in the peritoneal cavity and in the subcutaneous tissue (Fig. 2) (8).

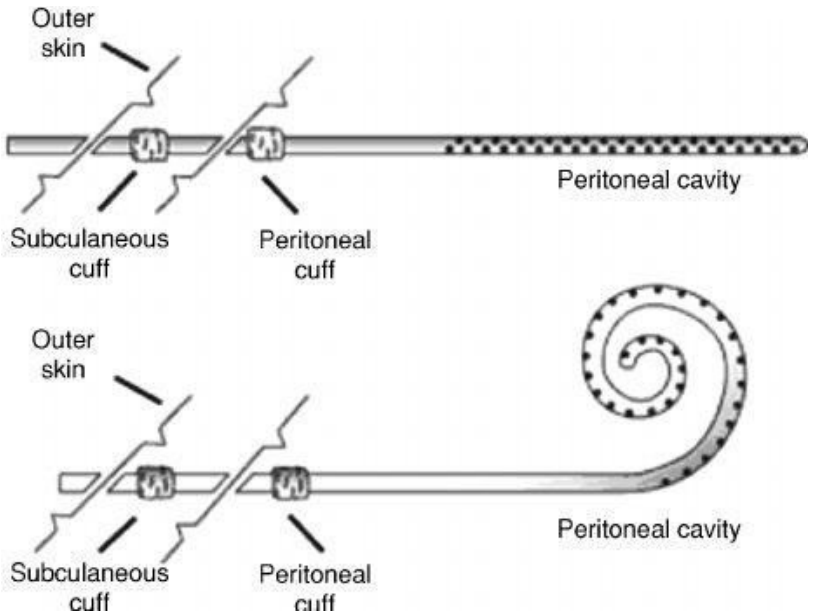

Fig. 2. A peritoneal catheter sketch (9)

Long-term peritoneal catheters used in chronic renal failure have a different design in both the intraperitoneal and extraperitoneal portions to prevent side effects and reduce coagulation (2).

Catheters are fenestrated silicone or polyurethane tubes with one or more Dacron sleeves. The intraperitoneal portion is fenestrated to allow the introduction of dialysis fluid. The distal portion of the catheter may be straight or curved. Curved catheters prevent fibrin obstruction. The extraperitoneal portion of the catheter has 1-2 Dacron sleeves. The migration of fibroblasts into the Dacron sleeves allows the anchoring of the catheter and provides a physical barrier against the leakage of dialysate as well as the entry of infectious agents. The portion between the two sleeves can be straight or it can be curved. Catheters with this permanently curved portion, also called a "swan neck", are produced to create a subcutaneous tunnel directed downward to reduce the risks of infection (Fig. 3) (11).

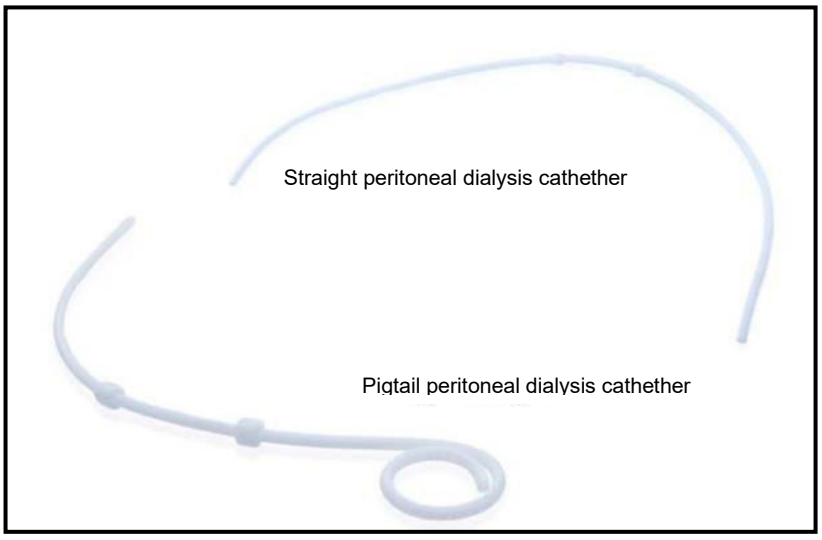

Fig. 3. Straight and pigtail peritoneal dialysis catheters (17)

In the case of peritoneal dialysis, studies have shown that with a single-sleeve catheter, peritonitis occurs in a shorter time and the rate of exit site infections is higher, although other studies have found no difference between the number of sleeves (7). Catheter insertion can be performed percutaneously, laparoscopically and through laparotomy, which offers the possibility of partial or total omentectomy. If peritoneal dialysis exceeds 24 hours, it is recommended to resort to omentectomy (12).

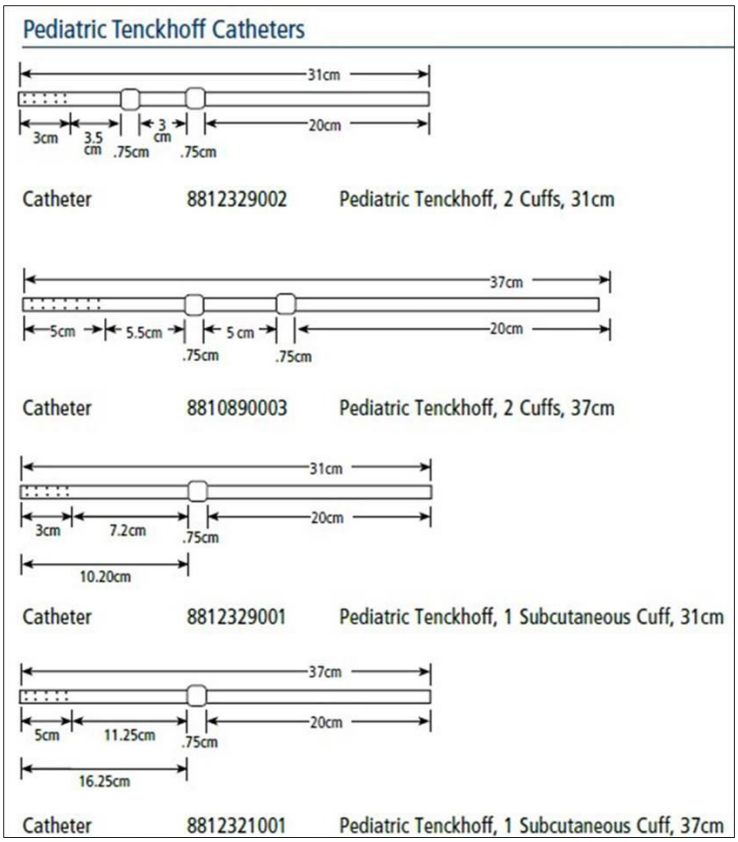

Fig. 4. Tenckoff straight catheter for chronic peritoneal dialysis (21)

Currently, there is a wide range of catheters that are used in the long term, but the most used are the "T" or straight Missouri and swan neck ones. Pediatric catheters are used in cats. The curved catheter is positioned in the groin area and the "T" one through the cranio-caudal oriented peritoneum. Catheter implantation is mandatory under aseptic conditions, in the operating room, in the area of the white line or paramedian, lateral to the umbilical scar. It is oriented caudally and positioned in the lower pelvis, and patency is checked before final fixation by introducing a small amount of dialysate. In the case of peritoneal dialysis, studies have shown that, with a single-sleeve catheter, peritonitis occurs in a shorter time and the rate of exit site infections is higher, although other studies have not found no difference between the number of sleeves (Fig. 4) (20).

The extraperitoneal portion of the catheter can be straight or have a bend between the two sleeves. Elbow or gooseneck catheters are manufactured to have

a subcutaneous tunnel that is directed downward to reduce the risk of catheter-related infections (18).

There are no studies conducted in veterinary medicine to evaluate the utility of a particular peritoneal dialysis catheter. In human medicine, the Tenckoff catheter is the most commonly used chronic peritoneal dialysis catheter. This silicone catheter has a straight extraperitoneal portion and either a straight or curved intraperitoneal portion with multiple holes in the distal end. The Tenckhoff catheter may have one or two Dacron cuffs (Fig.5) (19).

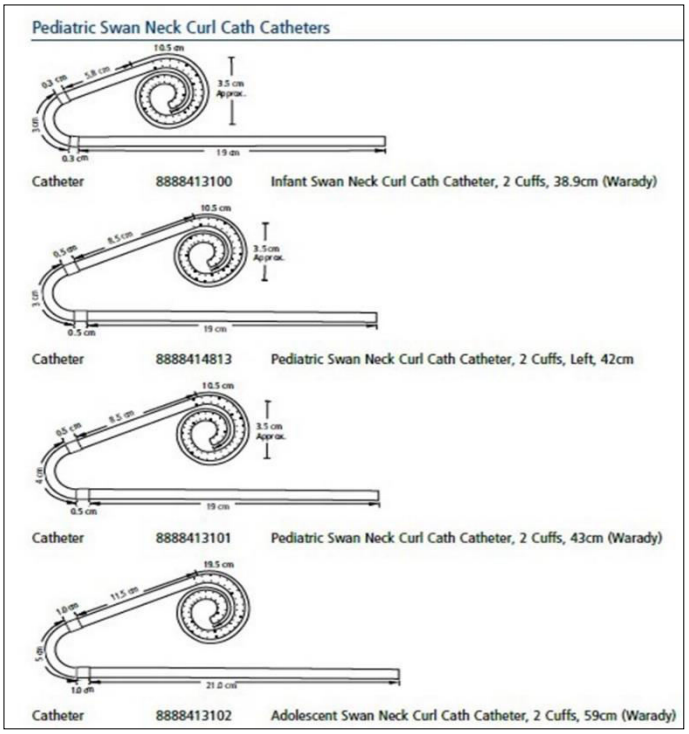

Fig. 5. Tenckoff catheter for chronic peritoneal dialysis (21)

Several types of catheters have been used in veterinary medicine to perform acute peritoneal dialysis (2).

The striated T-catheter was introduced in the 1990s and studies in dogs reported good results compared to the Tenckhoff catheter (coiled tube). This catheter is made of silicone and has two Dacron cuffs; this is a T-shaped catheter and is composed of long grooves. These grooves are designed to provide minimal resistance to the efflux and inflow of fluids while providing maximum protection

against omentum adhesion. The catheter is designed to be placed through the parietal peritoneum in a cranial-caudal direction. The catheter is designed to be 30 cm long, but can be cut to fit smaller patients. This catheter has been widely used in human medicine. Although its use has been reported in veterinary medicine, there are no clear studies evaluating its usefulness (9).

Other catheters used in veterinary medicine include Blake 15 Fr Surgical Drain, Swan Neck Straight or Curved, Missouri Catheter, 10 cm PD Catheter, Quinton Pediatric Peritoneal Dialysis Catheter, Dawson-Mueller Drain. All these catheters are surgically placed (Fig. 6, Fig. 7) (3).

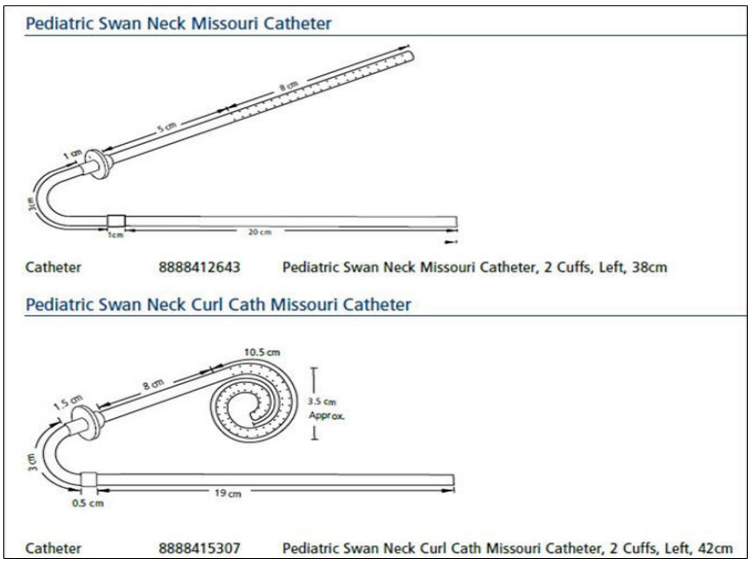

Fig. 6. Swan neck catheter for chronic peritoneal dialysis (21)

It is mandatory to mount the catheter under aseptic conditions, in the operating room, in the area of the white line or paramedian, lateral to the umbilical scar (7). The catheter is oriented caudally and is positioned in the lower area of the pelvis, in the bottom of the Douglas sac. Before the final fixation, the permeability is checked by introducing a small amount of dialysate (11). Many of the common complications associated with peritoneal dialysis in veterinary medicine are related to the catheter. Therefore, it is important to consider the type of catheter and its placement techniques when choosing this modality (16).

A recent study in healthy dogs describes a new method for implanting disc catheters for peritoneal dialysis through small incisions with very good results.

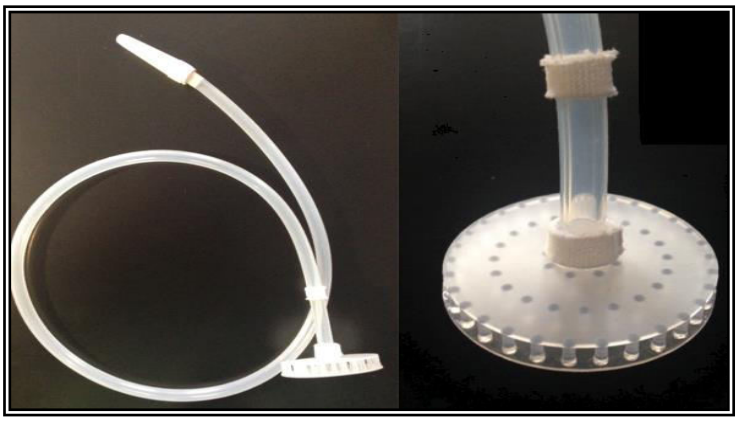

Fig. 7. Disk type catheter for peritoneal dialysis (17)

Simple trocar tube catheters can be placed in conscious animals using local anesthetics in major emergencies. The percutaneous cystotomy catheter (Cystofix Percutaneous Suprapubic Catheter Set) has also been shown to be used in veterinary medicine successfully in place of a peritoneal catheter in patients with renal disease (Fig. 8) (6).

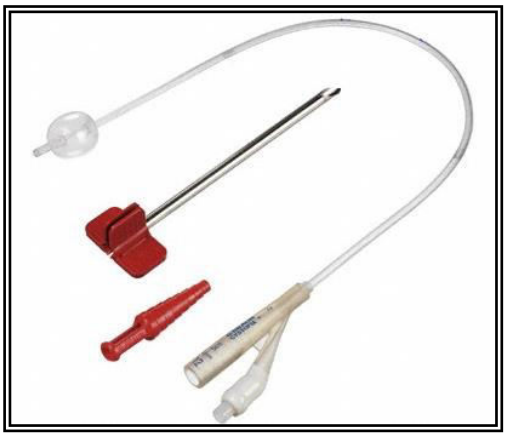

Fig. 8. Suprapubic Cystofix Percutaneous Catheter (17)

Peritoneal dialysis solutions

The specific composition of the dialysate is an important factor to consider when performing peritoneal dialysis. Dialysate solutions differ based on the buffer

component, electrolytes and/or osmotic agents used. The ideal peritoneal dialysis solution should achieve low-absorption clearance of osmotic agents, supply deficient electrolytes and nutrients, correct acid-base problems, inhibit the growth of microorganisms, and be inert to the peritoneum (3).

Dialysis buffer options historically have included lactate, bicarbonate, and acetate (14). To avoid precipitation and caramelization problems, bicarbonate-based solutions are presented in two-compartment bags. The chambers are mixed immediately before instillation of the dialysate into the peritoneum (10).

Commercially available dialysis solutions contain sodium, magnesium, calcium and chlorine in varying concentrations. Potassium is generally not included in dialysate solutions, but may be added if patients become hypokalemic during treatment (13).

Osmotic agents can be grouped into low molecular weight agents and high molecular weight agents. Low molecular weight agents that are used include glucose, glycerol, sorbitol, amino acids, xylitol and fructose. Xylitol is toxic to animals so, from the start, it is excluded (15).

Standard dialysis solution contains glucose as an osmotic agent. Glucose-based dialysate is present in three different concentrations: 1.5%, 2.5% and 4.25%. Dialysis performed to remove uremic toxins is generally done using a 1.5% solution (2). The use of a hypertonic glucose solution (4.25%) is reserved for overhydrated patients in whom the highly osmotic dialysate causes water to be removed from the body by osmosis (10).

Peritoneal dialysis can be performed using commercial dextrose-based dialysate solutions, or the dialysate can be formulated by adding dextrose to lactated Ringer's solution (18).

Glucose has been shown to be safe, effective, cheap and readily available. However, glucose can also be easily absorbed, leading to metabolic disorders such as hyperglycemia, hyperlipidemia, hyperinsulinemia, and obesity (8).

High molecular weight osmotic agents include polymers of glucose (polyglucose) such as icodextrin. Icodextrin is a glucose polymer soluble in water and starch, with a molecular weight of 16,800 Da. It is commercially available as a 7.5% solution in a milk buffer (11).

Icodextrin is an iso-osmotic solution (285 mOsm/kg) that produces ultrafiltration through its oncotic effect. Glucose polymers induce ultrafiltration through large pores through colloidal oncotic effects, compared to hyperosmotic dextrose solutions, which induce ultrafiltration through both small and very small pores (12). Absorption of icodextrin occurs through the peritoneal lymphatics, so it maintains its oncotic effect longer than dextrose-based solutions (14).

Icodextrin is used for long periods of time in continuous cyclic peritoneal dialysis in patients with ultrafiltration insufficiency and patients with diabetes mellitus. The use of icodextrin in veterinary medicine has not been investigated. It is usually recommended for critically ill patients to administer once daily (8-16 hours/overnight) and is alternated with standard dextrose solutions. This combination maintains fluid

removal using the dextrose solution and optimizes ultrafiltration with the icodextrin solution. More recently, amino acids have been used as osmotic agents, mainly due to a presumed positive effect on nutritional status (5).

The best known and used peritoneal dialysis solution with amino acids is Nutrineal (Nutrineal™ PD4) (peritoneal dialysis solution with 1.1% amino acids) which is intended for intraperitoneal administration only, replacing one or two dextrose-containing shifts per day, as part of the daily dialysis regimen for patients with protein malnutrition. Adequate dialysis should be performed prior to initiation of treatment with Nutrineal™ PD4 (4). The recommended total daily dose of protein is greater than or equal to 1.2 g/kg body weight for dialysis patients. The 2-liter bag of Nutrineal™ PD4 contains 22 g of amino acids which corresponds to 1.2 g/kg body/24 hours (100% of the daily protein requirement) for a 17.5 kg dialysis patient (16).

Several other substances are often added to peritoneal dialysis solutions as needed. Insulin can be added to dialysate solutions in diabetics to help control hyperglycemia (11).

There are peritoneal dialysis solutions that contain amino acids, to supplement the amino acid deficiency suffered by the dialysis patient (Fig. 9) (9).

Fig. 9. Comparative aspect of unicameral and bicameral dialysis bags (22)

"**Home made**" peritoneal dialysis fluid recipes can also be used (8).

"**Home made**" preparations contain Sodium Chloride 0.45% or 0.9% or Lactated Ringer, depending on the needs of the patient and the addition of dextrose (10).

Dextrose in a concentration of 1.5% lends itself to a dialysate solution and can be achieved by adding 30 ml of 50% dextrose to a liter of lactated Ringer or 50 ml to obtain a 2.5% solution. To this is added:

- magnesium (72 mg/liter → 1.5 meq/l);
- sodium bicarbonate (30-45 meq/liter for alkalinization);

- heparin (before infusion, 1000 IU/l);
- antibiotics (if peritonitis is suspected);
- potassium (in hypokalemia, 4 meq/l).

In case of hypercalcemia or hyperphosphatemia, we use solutions without calcium or phosphorus (NaCl 0.9% and Glucose). In case of hyperkalemia, we do not use Ringer, but NaCl 0.9% and Glucose (19).

Antibiotics may be added to the dialysate solution to treat peritonitis, although routine use of antibiotics is discouraged. The cephalothin used in an initial dose of 1g/liter of dialysate, a dose that will be adjusted later to 350mg/liter for 7-14 days, gave the expected results (8).

Heparin is frequently added to the dialysate solution to prevent fibrin sheath formation on the peritoneal dialysis catheter. The addition of heparin to the peritoneum does not lead to systemic anticoagulation. 500 to 1000 units of heparin may be added to each liter of dialysate to reduce clotting in the catheter lumen (18).

Conclusions

The number of cases of kidney disease among felines is constantly increasing. In cases diagnosed with chronic kidney disease, due to the slow and progressive evolution (intervals of years) and the erased symptomatology in the early stages, many patients are presented to the veterinarian after the kidney function is reduced to 25% of the normal value.

Chronic kidney disease (CKD) is the result of a long-term nephropathy involving the irreversible reduction of active nephrons, compensated by the hypertrophy of other nephrons involving a drastic decrease in glomerular filtration. The condition has a staged evolution characterized in the end by anuria.

Uremia, kidney damage and acute poisoning are some of the common medical problems in cats. In acute kidney injury (AKI) there is a rapid decrease in glomerular filtration caused by renal ischemia or toxic damage at this level. Rapid diagnosis and the institution of treatment such as peritoneal dialysis can lead to a full recovery.

Peritoneal dialysis is an alternative method of treating chronic kidney disease when medical or dietary treatment is insufficient (elevated levels of creatinine and urea, hyperkalemia, hyperphosphatemia, hypercalcemia or metabolic acidosis that does not yield to treatment). This treatment method has become a widely used practice for the removal of dialyzable (endogenous and exogenous) toxins, thus occupying an important place in the therapy of chronic kidney disease in cats, especially in geriatric patients.

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References

1. **Awad, A.S., Rouse, M., Huang. L.,** Compartmentalization of neutrophils in the kidney and lung of following acute ischemic kidney injury, Kidney International, 2009, 75, 689-698.
2. **Crisp, M.S., Chew, D.J., Dibartola, S.P., Birchard, S.T.,** Peritoneal dialysis in dogs and cats: 27 cases (1976-1987), Journal of the American Veterinary Medical Association, 1989, 195, 9, 1262-1266.
3. **De Vriese, A.S., Mortier, S., Cornelissen, M., Palmans, E., Vanacker, N.J., Leyssens, A., Lameire, N.H.,** The effects of heparin administration in an animal model of chronic peritoneal dialysate exposure, Peritoneal dialysis international, 2002, 22, 5, 566-572.
4. **Diaz-Buxo, J.A.,** What is the role of automated peritoneal dialysis and continuous flow peritoneal dialysis?, Contributions to Nephrology, 2003, 140, 264-721.
5. **Fiaccadori, E., Regolisti, G., Cabassi, A.,** Specific nutritional problems in acute kidney injury, treated with non-dialysis and dialytic modalities, NTD Plus Nephrology Dialysis Transplantation, 2010, 3, 1-7.
6. **Golper, T., Churchill, D., Burkart, J.,** Clinical practice guidelines for peritoneal dialysis adequacy, American Journal of Kidney Diseases, 1997, 30, 2, S67-136.
7. **Holowaychuk, M.K., Marks, S.L.,** Peritoneal Dialysis, Critical Care Medicine Section Department of Clinical Sciences, North Carolina State University, 2011.
8. **Kushwaha, R., Singh, N.,** Peritoneal Dialysis in animals- a review, Journal of Veterinary Medicine, 2008, 7, 1, 1-9.
9. **Peppelenbosch A., Kuijk W., Bouvy N., Sande F., Tordoir, J.,** Peritoneal dialysis catheter placement technique and complications, Plos One, 10, 1093, 2008.
10. **Viţalaru, B.A.,** Dializa peritoneală la pisică, Revista Catlife, București, 2014, 6.
11. **Viţălaru, B.A, Bălăşcău, Ş.B., Chitoroiu, A.L., Păun S.C., Ivașcu, C., Bociu, N.A., Munteanu, R., Toader, C.,** Choosing the dialyzer type In order to perform optimum hemodialysis in dogs, Journal of Biotechnology, 2016, 231.
12. **Viţălaru, B.A., Bălăşcău, Ș.B.,** Antifreeze Acute Intoxication: Hemodialysis in Two German Sheppard Dogs, Bulletin UASVM Veterinary Medicine, Bucuresti, 2015, 72, 2.
13. **Viţălaru, B.A., Bociu, N.A., Bălăşcău, Ş.B., Toader, C., Toader, M., Drăghici, M., Ștefănescu, A.,** The influence of hemodialysis on hemoglobin in dogs with acute renal injury, Journal of Biotechnology, 2017, 256, S91-S92.
14. **Viţalaru, B.A.,** Dializa peritoneală în medicină veterinară, Revista Veterinaria, 2014, 16, 18-20.
15. **Viţălaru, B.A.,** Hemodializa vs. dializa peritoneală la carnivorele domestice, Practica veterinară, AMVAC, 4, 2014, 49, 2014.

16. **Viţălaru, B.A., Micşa, C., Bîrţoiu, I.A.,** Peritoneal dialysis: key therapy in kidney failure in small sized dogs, Bulletin UASVM Veterinary Medicine Bucuresti, 2014, 71, 2.
17. **Viţălaru, B.A., Ștefănescu, A.,** Bolile congenitale renale la feline, Revista Catlife, 2017, VI, 12, 2.
18. **Viţălaru, B.A., Ștefănescu, A.,** Dializa peritoneală la animalele de companie, Ed. Printech, București, 2017.
19. **Viţălaru, B.A., Ştefănescu, A.,** Protocoale de combatere a anemiei la pacienţii cu insuficienţă renală, revista Practica Veterinară, București, 2015, 20, 3.
20. **Viţălaru, B.A.,** Urgențe în medicina animalelor de companie vol. I, Editura Elisavaros, București, 2016.
21. *** http://www.covidien.com
22. *** www.fmc-romania.ro

RESEARCH ON THE EFFECT OF BIOLOGICAL EXTRACTS IN THE TREATMENT OF ALLERGIC DERMATITIS IN DOGS

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Summary

Recently, in a previous study regarding the *in vitro* antibacterial efficacy of plants from the Fabaceae family, we reported high antibacterial efficacy especially on *Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa and Streptococcus pyogenes stains*. This study was therefore, designed to test the *in vivo* efficacy of two plants belonging to the Fabaceae family, namely *Robinia pseudoacacia* and *Melilotus officinalis*. A number of 30 dogs, diagnosed with atopic dermatitis, were chosen to test the antibacterial effect of the extracts. The efficacy of the extracts was monitored by evaluating the total germ count and total staphylococci isolated from the skin. After testing the *M. officinalis* and *R. pseudoacacia* extracts, a decrease in the microbial load was observed. In this sense, the extract of *R. pseudoacacia* had the strongest effect of reducing microorganisms, followed by the extract of *M. officinalis* and the extract obtained from the mixture of the two plants. Regarding the reduction of staphylococci, the *M. officinalis* extract had the strongest effect in reducing the bacterial load, followed by the mixture extract and the *R. pseudoacacia* extract. The study revealed that the plant extracts can reduce the bacterial load, however the reduction level was too low to be considered an effective antibacterial solution.

Keywords: *Melilotus officinalis; Robinia pseudoacacia*; microbiological activity.

Presently, bacteria strains with multiple antibiotic resistance are becoming more prevalent, presenting a serious issue for both human medicine and veterinary medicine. As a result, treatment of patients, particularly those who are immunocompromised, is sometimes difficult due to the poor potency of the treatments and the onset of diseases brought on by free radicals, despite the fact that there are an increasing number of antibacterial and antifungal chemicals available. This circumstance, together with the unintended consequences of some medications, is a severe medical issue, and it is crucial to find alternative sources of antibacterial and antifungal medicines.

In allergic animals, the immune system reacts with the high production of pro-inflammatory cells and molecules, which will lead to skin lesions accompanied by itching. In the case of intense itching, a vicious circle will be created, in which the skin lesions will persist for a long time, thus, secondary bacterial and fungal infections will occur (13).

Secondary infections usually attract the attention of the owner and the clinician as they are considered, most of the time, to be the primary cause of pruritus, not considering the possibility of an allergic disease. Also, these secondary lesions are generally recurrent, returning to a subsequent contact of the animal with the

causative allergen. In these situations, the treatment used by clinicians is usuallysymptomatic, thus, some animals can end up showing marked resistance to the usual antibiotics (4).

OMS reports over the past two years confirm a sharp increase in the number of pathogenic microorganisms that have evolved multiple antibiotic resistances (22). Unfortunately, the pharmaceutical industry cannot overcome the emergence of microorganisms with widespread resistance to antimicrobial substances by producing new antibiotics.

This study is based on previous studies of the in vitro antimicrobial, antifungal, and antioxidant activities of four extracts obtained from the flowers of several species belonging to the Fabaceae family, which are commonly found in Romanian western floral species. In this study, it was observed that *Melilotus officinalis* (MO), *Coronilla varia* (CV), *Ononis spinosa* (OS), and *Robinia pseudoacacia* (RP) have antimicrobial effects, particularly on the stains of *E. coli*, *S. typhimurium*, *P. aeruginosa,* and S. *pyogenes*. Our study focused on evaluating the *in vivo* antimicrobial effects of three extracts, specifically *M. officinalis, R. pseudoacacia*, and a combination of those two plants (16).

Materials and methods

Selection of the dog batch

A total of 30 dogs with atopic dermatitis were selected and divided equally into the following three groups to assess the extracts antibacterial effects *in vivo*:

M. officinalis extract was used in group 1, *R. pseudoacacia* extract was used in group 2, and a combination of the two plants was used in group 3.

Anti-inflammatories and antibiotics were not administered to any of the study's canine participants. During the research time, it was also avoided to wash the animals using topical antibacterial solutions.

The efficacy of the extracts was monitored by evaluating the total germ count (TGC) and the total number of staphylococci isolated from the skin.

The chosen dogs had the highest staphylococcal load during the initial check and a total germ count of at least 500 cfu.

The collection of samples, for the evaluation of the microbial load, was carried out with the help of a sterile swab, the samples being collected from the skin of the abdomen, from a well-defined area of 25 cm^2. Serial dilutions were performed to obtain a representative number of colony-forming unit (cfu), between 30-300, and PCA medium was used for the evaluation of the total number of germs and Chapman's medium for the isolation of staphylococci. The inoculated plates were incubated for 24 hours at a temperature of 37^{o}C, then colonies were counted and identified based on morpho cultural characters (Fig. 1).

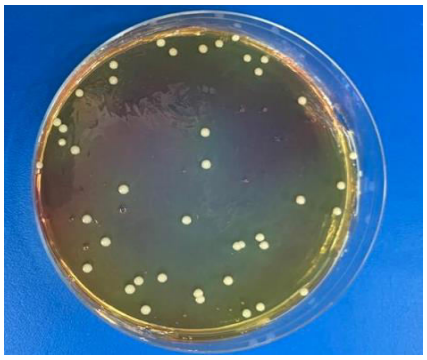

Fig. 1. *Staphylococcus* colonies on Chapman medium

Preparation of extracts

To perform the experiments, the aerial parts of the plant species under study were collected during the flowering period, from the wild flora of Timișoara. About 500 g of fresh material was collected and used from each species.

The plants were air-dried at 25°C and ground using a grinder (Blixer 3; Retsch Technology GMbH, Haan, Germany) to a fine powder. A 10% extract was prepared from the powder obtained (50 g of powder to 500 ml solution of 30% ethanol, 70% water, and a 1:1 mixture in the case of the combined extract). The obtained preparation was maintained for 60 min on the magnetic stirrer, being then filtered and distributed in containers (Fig. 2).

Fig. 2. Obtained plant extracts

Treatment

The treatment was administered at home by the owners twice a day for five days. Owners were instructed to spray 20 ml/day of the extract on the main affected areas, namely the axillary region, thoracic and inguinal areas. The dogs wore

Elizabethan collars and were not permitted to lick the extract. Samples were again gathered on the sixth day for the microbiological analysis.

Results and discussions

Microbiology analyses of the sanitation swabs collected after the treatment indicated a decrease in the microbial load. In this sense, the extract of *M. officinalis* had the strongest effect of reducing microorganisms, followed by the extract of *R. pseudoacacia* and the extract obtained from the mixture of the two plants. Thus, following the administration of *M. officinalis* extract, a reduction in the total number of germs in the group 1 of dogs was observed from 1160 x 10^4 ± 135 x 10^4 cfu/ml to 1137 x 10^4 ± 207 x 10^4 cfu/ml. The *R. pseudoacacia* extract reduced the total germ count of group 2 from 851 x 10^4 ± 101 x 10^4 cfu/ml to 826 x10^4 ± 100 x 10^4 cfu/ml, and in group 3 the mixture extract reduced the total germ count from 838 x 10^4 ± 54 x 10^4 cfu/ml to 817 x 10^4 ± 55 x 10^4 cfu/ml (Fig. 3).

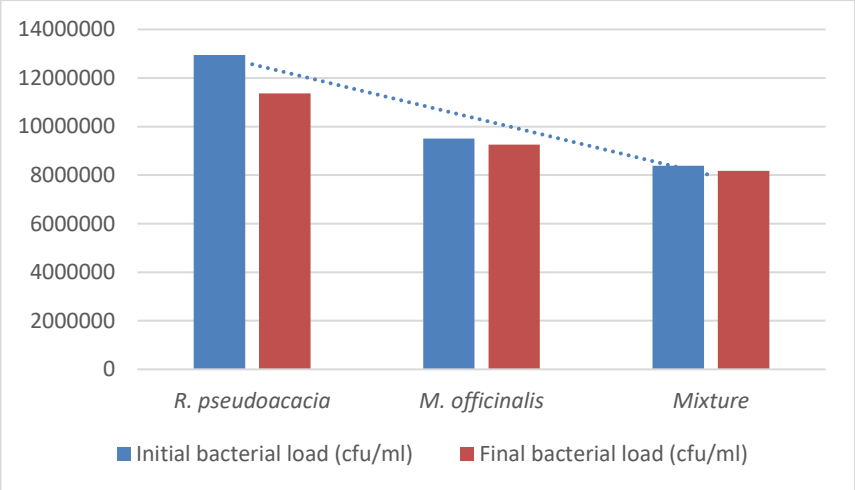

Fig. 3. Effect of extracts on TGC

Regarding the reduction of staphylococci, the *M. officinalis* extract had the strongest effect in reducing the bacterial load, followed by the mixture extract and the *R. pseudoacacia* extract (Fig. 4). Thus, the extract of *M. officinalis* reduced the number of staphylococci from 667 x 10^4 ± 840 x 10^4 cfu/ ml to 649 x 10^4 ± 845 x 10^4 cfu/ml, the mixture extract reduced the number of staphylococci from 816 x 10^4 ± 454 x 10^4 cfu/ml to 802 x 10^4 ± 451 x 10^4 cfu/ml, and the *R. pseudoacacia* extract from 710 x 10^4 ± 790 x 10^4 cfu/ml to 698 x 10^4 ± 790 x 10^4 cfu/ml.

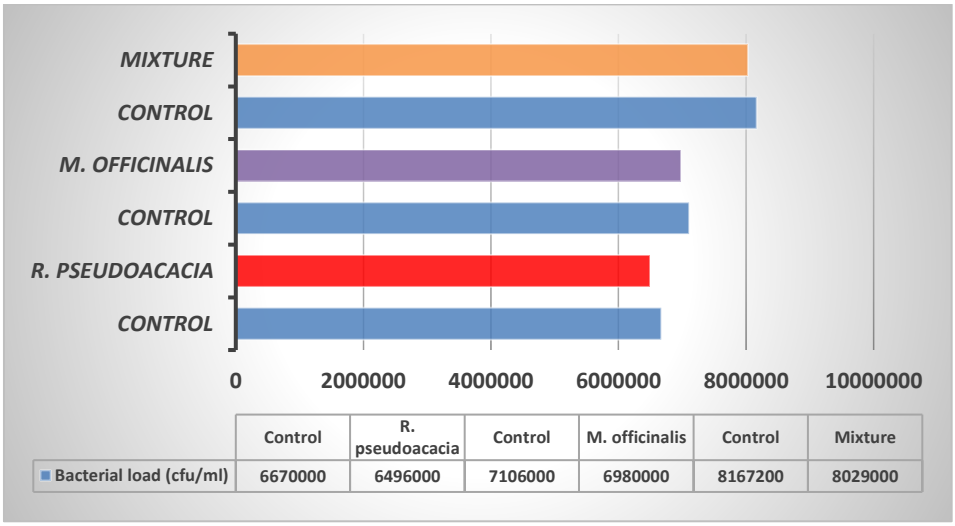

Fig. 4. Effect of extracts on staphylococci

M. officinalis extracts are known for their positive effects on insect bites, venous circulatory disorders, liver disorders (8, 14), have an antibacterial (3, 15, 16, 17), and antiparasitic activity (1). *R. pseudoacacia* extracts have antacid, antifungal, purgative, and emmenagogue properties, as well as being antibacterial (6, 7, 16, 18), antiviral (9), and antiparasitic (10).

At the same time, many studies are beginning to show the anti-inflammatory potential of plants with high polyphenol content, such as the extracts of *M. officinalis* and *R. pseudoacacia* used in this study (2, 5, 11, 12, 18, 19, 20, 21), but the *in vivo* antimicrobial activity of the extracts has not been thoroughly studied. Our study is the first in Romania to look at the antimicrobial activity of the two extracts *in vivo*.

Conclusions

The antibacterial potential of *Melilotus officinalis* extracts and *Robinia pseudoacacia*, against Gram positive bacteria, suggests that they can be viable alternatives to synthetic products in the treatment of allergic diseases.

Our research on the antimicrobial potential of *R. pseudoacacia* and *M. officinalis* revealed that plant extracts can reduce the bacterial load by lowering the total germ count and staphylococci count on the skin of dogs in a short period of time. Further in vivo studies with different extract concentrations and longer periods of exposure are required to evaluate and identify better solutions that can compete with common antiseptics used in veterinary and human medicine.

References

1. **Acharya, J., Hildreth, M.B., Reese, R.N.,** In vitro screening of forty medicinal plant extracts from the United States Northern Great Plains for anthelmintic activity against *Haemonchus contortus*, Veterinary Parasitology, 2014, 201, 1-2, 75-81.
2. **Akiyama, H., Sato, Y., Watanabe, T., Nagaoka, M.H., Yoshioka, Y., Shoji, T., Kanda, T., Yamada, K., Totsuka, M., Teshima, R., Sawada, J., Goda, Y., Maitani, T.,** Dietary unripe apple polyphenol inhibits the development of food allergies in murine models, FEBS Letters, 2005, 579, 20, 4485-491.
3. **Al-Snafi, A.E.,** Therapeutic properties of medicinal plants: a review of their antibacterial activity (part 1), International Journal of Pharmacology and Toxicology, 6, 3, 137-158.
4. **Chan, S.K., Leung, D.Y.M.,** Dog and Cat Allergies: Current State of Diagnostic Approaches and Challenges, Allergy Asthma Immunol Res., 2018, 10, 2, 97-105.
5. **Bojor, O., Popescu, O.,** Traditional and modern phytotherapy, Fiat Lux Publishing house, Bucharest, 2003.
6. **Dobrányi, S., Dancs, G., Posta, K., Fülöp, L.,** Growth inhibitory effects of bioactive metabolites in wood extracts of *Robinia pseudoacacia* L. inhibit the growth of various microorganisms, Journal of Universal Science Online, 2014, 1, 1, 28-34.
7. **Forni, C., Facchiano, F., Bartoli, M., Pieretti, S., Facchiano, A., D'Arcangelo, D., Norelli, S., Valle, G., Nisini, R., Beninati, S.,** Beneficial role of phytochemicals on oxidative stress and age-related diseases, BioMed Research International, 2019, 2019, 8748253.
8. **Grigorescu, E., Ciulei, I., Stănescu, U.,** Phytoterapic Index, Medicala, Bucuresti, 1986.
9. **Guo, H., Wan, X., Niu, F., Sun, J., Shi, C., Ye, J.M, Zhou, C.,** Evaluation of antiviral effect and toxicity of total flavonoids extracted from *Robinia pseudoacacia* cv. Idaho, Biomed Pharmacotherapy, 2019, 118, 109335.
10. **Katiki, L.M, Ferreira, J.F, Gonzalez, J.M, Zajac, A.M, Lindsay, D.S, Chagas, A.C, Amarante, A.F.,** Anthelmintic effect of plant extracts containing condensed and hydrolyzable tannins on Caenorhabditis elegans, and their antioxidant capacity, Veterinary Parasitology, 2013, 192, 1-3, 218-27.
11. **Khosroyar, S., Arastehnodeh, A.,** Comparison of anti-inflammatory and antioxidant capacity of alcholic extraction of fraxinus fxcelsior and melilotus officinalis plant, Plant Archives, 2018, 18, 1, 443-448.
12. **Lee, M., Kim, S., Kwon, O. K., Oh, S. R., Lee, H. K., Ahn, K.,** Anti-inflammatory and anti-asthmatic effects of resveratrol, a polyphenolic stilbene, in a mouse model of allergic asthma, International immunopharmacology, 2009, 9, 4, 418-424.
13. **Martin, S.F.,** The role of the innate immune system in allergic contact dermatitis, Allergologie Select, 2017, 11, 39-43.

14. **Menković, N., Šavikin, K.; Tasić, S., Zdunić, G., Stešević, D., Milosavljević, S., Vincek, D.,** Ethnobotanical study on traditional uses of wild medicinal plants in Prokletije Mountains (Montenegro), Journal of Ethnopharmacology, 2011, 133, 97-107.
15. **Mladenović, K., Grujović, M., Stefanović, O., Vasić, S., Čomić, L.,** Antimicrobial, antioxidant and antibiofilm activity of extracts of *Melilotus officinalis* (L.) pall, Journal of Animal and Plant Sciences, 2016, 26, 1436-1444.
16. **Obistioiu, D., Cocan, I., Tîrziu, E., Herman, V., Negrea, M., Cucerzan, A., Neacsu, A.G., Cozma, A.L., Nichita, I., Hulea, A., Radulov, I., Alexa, E.,** Phytochemical profile and microbiological activity of some plants belonging to the Fabaceae family, Antibiotics (Basel), 2021, 10, 6, 662.
17. **Safarpour, A.R., Kaviyani, F., Sepehrimanesh, M., Ahmadi, N., Koohi Hosseinabadi, O., Tanideh, N., Showraki, N.,** Antioxidant and anti-inflammatory effects of gel and aqueous extract of *Melilotus officinalis* L. in induced ulcerative colitis: a Rattus norvegicus model, Iranian Journal of Colorectal Research, 2015, 3, 2, e29511.
18. **Semkina, O.A.,** Ointments, gels, liniments, and creams containing phytopreparations (a review), Pharmaceutical Chemistry Journal, 2005, 39, 7, 369-374.
19. **Shenefelt, P.D.,** Herbal Treatment for dermatologic disorders. In: Herbal Medicine: Biomolecular and Clinical Aspects, 2nd ed. CRC Press/Taylor & Francis, Boca Raton (FL), 2011.
20. **Singh, A., Holvoet, S., Mercenier, A.,** Dietary polyphenols in the prevention and treatment of allergic diseases, Clinical & Experimental Allergy, 2011, 41, 1346-1359.
21. **Zhao, G.C., Yuan, Y.L., Chai, F.R., Ji, F.J.,** Effect of *Melilotus officinalis* extract on the apoptosis of brain tissues by altering cerebral thrombosis and inflammatory mediators in acute cerebral ischemia, Biomed Pharma, 2017, 89, 1346-1352.
22. ***https://www.who.int/news/item/17-01-2020-lack-of-new-antibiotics-threatens-global-efforts-to-contain-drug-resistant-infections

PRELIMINARY RESEARCH ON THE ECONOMIC ADVANTAGES AND DISADVANTAGES OF HYBRIDS DERIVED FROM THE CROSSING OF TURCANA AND TSIGAI BREEDS

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Summary

Due to the growing demand for meat on the market, breeders must find the easiest economic methods that they can implement in their farms. In sheep, one such method is the hybridization of compatible breeds and observing if this can be satisfactory. The study takes into account the *Turcana* and *Tsigai* breeds, respectively the comparison of the production indicators between the *Turcana* lambs and the *Turcana x Tsigai* hybrid lambs (f1). The study took into account 2 groups of lambs, 20 each, and the differences such as weight at calving and average daily gain were monitored. Among the first advantages observed, we can mention the higher weight of the hybrid lambs at calving as well as a higher average daily gain in the first weeks, and as disadvantages, we can mention the occurrence of dystocia at calving due to the large size of the hybrid lambs.

Keywords: lambs, average daily gain, hybrids, *Turcana, Tsigai*.

Sheep breeding is one of the most important branches of agriculture in Romania. Sheep are raised mainly for meat production, but they are also raised for milk, wool and skins production (3). In Romania, as in other Eastern European countries, sheep are raised more for meat production (15), compared to countries in the Mediterranean basin, where milk production is more important (20).

The most predominant sheep breed in our country is represented by the *Turcana*, followed by the *Merino*, *Tsigai, Ratca* breeds etc. (17).

Traditionally, due to Easter customs, the biggest demand on the market in Romania is for suckling lambs (3). Due to the high demand for meat on the market, breeders have to find the most useful methods to cope (4, 9, 13, 16, 23).

Among the solutions found by animal breeders to improve the performance of the animals they raise are: improving the feed ration (2, 11, 19) and hybridizing their breeds with other breeds (1, 8, 17, 18).

In our study, we followed the productive advantages of the lambs produced from the hybridization of the *Turcana* breed with the *Tsigai* breed, specialized in meet production.

Materials and methods

Experimental protocol

The study began on two groups of females of 25 heads each from the *Turcana* breed, in a sheep farm in Timiș county. The groups of females were selected objectively, as similar as possible in terms of age, height and weight of the animals. Each group of females was assigned a different male during the breeding period, namely: group 1 was assigned a *Turcana* male and group 2 a *Tsigai* male. At calving, 20 lambs were selected from each group of females and monitored for calving weight, one-month weight and average daily gain.

During the breeding season, as well as during the gestation period, the animals were kept on pasture, and after calving, they were kept in closed stables. During the entire period of the experiment, all lambs in both groups consumed milk from their mothers and benefited from field hay, concentrate feed and water at discretion. Adult sheep consumed about 300 g of corn/head daily, and field hay and water were ad libitum.

During the experiment, all the lambs were coprological tested with the Willis method (5), in order to exclude the possibility of parasitism that could influenced the final results.

Methods

The lambs were weighed individually, with an electronic scale, at the time of calving and one month after calving.

Statistical calculations were performed in Microsoft Excel, and statistical interpretation was performed using the Student's test (TTEST).

Results and discussions

The results of lamb weighings at birth and at one month of age for both groups are attached in Tables 1 and 2.

Table 1

Weight of *Turcana* lambs at calving and 28 days

Group 1 – *Turcana* lambs		
	Day 0	Day 28
Total kg. group	102	296.8
ADG (kg.)	0	0.348
Mean	5.1	14.84
Standard Deviation	0.75742	1.28079
Standard Error	±0.16936	±0.28639

Table 2

Weight of *Turcana* X *Tsigai* hybrid lambs at calving and 28 days

Group 2 – *Turcana X Tsigai* hybrid lambs		
	Day 0	Day 28
Total kg. group	117.4	338
ADG (kg.)	0	0.394
Mean	5.87	16.9
Standard Deviation	0.68063	1.98468
Standard Error	±0.15219	±0.44379

The difference in weight evolution of the lamb groups during the experiment can be seen in Fig. 1.

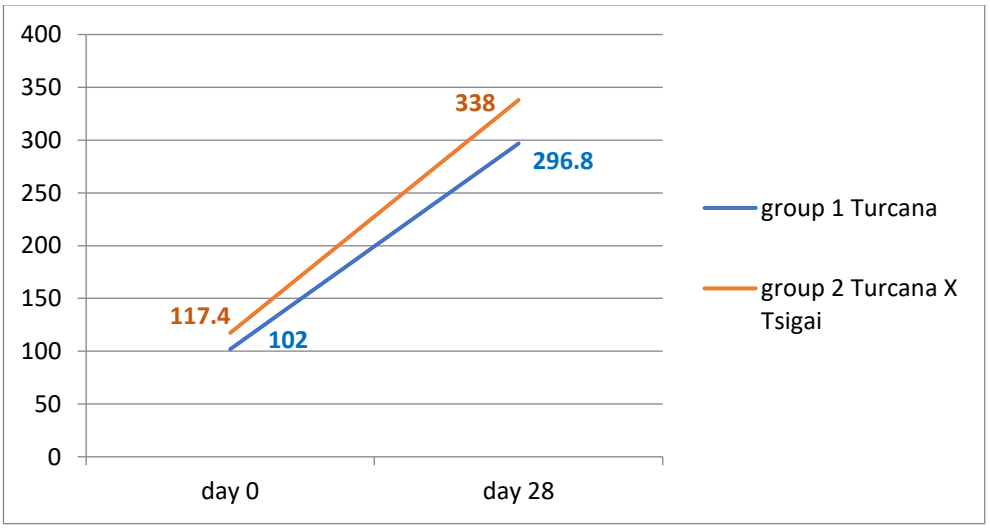

Fig. 1. The evolution of the weight (kg.) of the lamb groups

According to the Ttest the "p" value is less than 0.05 both on day 0 between the groups and at the end of the experiment (0.00084 on day 0, respectively 0.00019 on day 28) which demonstrates a significant difference between the two groups of lambs, although they were raised under the same conditions.

From the moment of birth, the hybrid lambs presented a greater weight than those of the Turcana breed, a fact that could be an advantage during the

experimental period, as they also had a greater weight at the end of the experiment.

Similar studies in the country have shown advantages in terms of body weight of hibrid lambs compared to *Turcana* lambs (6, 7, 14, 21, 22). Also, in other regions of the world, the increase of productivity has been demonstrated as a result of crossbreeding (10, 12).

Due to the greater development of the hybrid lambs, some sheep suffered dystocia at calving and required assistance, especially primiparous sheep.

The differences in average daily gain between lambs in the same group were due to the different amount of milk produced by each female.

Conclusions

Crossing the *Turcana* breed with the *Tsigai* breed brought a benefit in terms of calving weight and average daily gain of lambs.

Hybridization of primiparous females can lead to the occurrence of dystocia and thus it is recommended to hybridize multiparous females with *Tsigai* rams to avoid this pathology.

More studies are needed to be able to track the economic benefit brought by these hybridizations.

References

1. **Borzan, M.M., Cîmpean, A., Pusta, D., Bogdan, L., Tabaran, A., Pall, E., Mate, A., Cenariu, M., Pasca, I.,** Body Weight Evolution of Tsigai and Tsigai and Berrichon du Cher Crossbred Lambs, Bucharest Chemistry Magazine, 2019, 8, 70.
2. **Cireșan, C.A., Cărpinișan, L., Luca, I., Sîrbu, C.B., Imre, M., Hotea, I., Marin, A.M., Onmaz, A.C., Dărăbuș, Gh.,** Alternative recipes for improving the average daily gain in lamb, Lucrări Ştiinţifice Medicină Veterinară, 2022, LIV, 2, 46-52.
3. **Dărăban, S.,** Study concerning some carcass traits in young sheep fattened in different systems. Bulletin USAMV-CN, Animal Sciences and Biotechnologies, 2008, 65, 1-2, 161-166.
4. **Dărăbuș, Gh., Oprescu, I., Morariu, S., Mederle, N., Ilie, M.S.,** Practical guide in parasitic diseases volume I, Agoprint, Timisoara, 2013.
5. **Găvojdian, D., Cziszter L.T., Sossidou, E., Păcala, N.,** Improving performance of Zackel sheep through cross-breeding with prolific Bluefaced Leicester under semi-intensive and extensive production systems, Journal of Applied Animal Research, 2013, 41, 4, 432-441.
6. **Găvojdian, D., Sauer, M., Păcala, N., Pădeanu I., Voia, S.,** Improving Growth Rates in Turcana Indigenous Sheep Breed Using German Blackheaded Mutton Rams, Animal Science and Biotechnologies, 2011, 44, 2,

379-382.
7. **Ghiță, E., Lazăr, C., Pelmus, R., Voicu, I.,** Comparative research on the fattering aptitudes of growing lambs of local Romanian breeds, Biotechnology in Animal Husbandry, 2010, 26, 1-2, 13-20.
8. **Ilişiu, E., Dărăban, S., Gabi-Neacşu, M., Ilişiu, V.C., Rahmann / Landbauforschung, G.,** Improvement of lamb production in Romania by crossbreeding of local Tsigai breed with high performance breeds, Agriculture and Forestry Research 4, 2010, 60, 259-266.
9. **Marinchenko, T.E.,** Ways of Increasing the Efficiency of Sheep Breeding in the Russian Federation, Earth and Environmental Science, 2022, 949, 1-6.
10. **Mavrogenis, A.P.,** Breeding systems and selection strategies for sheep improvement in Cyprus. Zaragoza: CIHEAM Cahiers Options Méditerranéennes, 1995, 11, 17-26.
11. **MCManus, M., Gomes, E.F., Paim, T.P., Louvandini, H., Dallago, B., Borges, B.O., Zoran, A., Lima, P.T.,** Effect of supplementary milk feeding on growth and survival of Santa Inês lambs, Cienc. anim. bras., Goiânia, 2014, 15, 4, 451-457.
12. **Mitchell, J.,** Crossbreading in Sheep - Issues and Options with a Focus on Early Lamb Systems, Kellogg Project, 2000.
13. **Murat, L., Ionescu, A., Lucaci, E., Raşcu, D., Dâchi, H., Bahrim, V.,** Aptitudinea pentru producția de carne a mieilor hibrizi comparativ cu rasele parentale. Lucrări Științifice ICPCOC Palas – Constanța, 1994, VII, 141-148.
14. **Nagy, B., Vlaic, A., Mireșan, V., Odagiu, A.,** Research Concerning the Fattening Performances of Norwegian White x Turcana Hybrids Compared to Turcana Pure Breed - Note II - Bulletin UASVM Animal Science and Biotechnologies, 2010, 67, 1-2.
15. **Pădeanu, I.,** Meat production in sheep, Ed. Mirton, Timisoara, 2010.
16. **Pădeanu, I.,** Technical evaluation and genetical improvement of sheep productions, Ed. Mirton, Timisoara, 2003.
17. **Popa, D., Rusu, M., Pădeanu, I., Cotirlea, D., Voia, S.O., Sprijinean, D., Bozdog, C.,** Results Regarding Body Weight and Average Daily Gain in Turcana and Crossbred Suffolk x Turcana Lambs, Bulletin UASVM, Veterinary Medicine 2011, 68, 1.
18. **Rodríguez, A.B., Bodas, R., Landa, R., López-Campos, Ó, Mantecón, A.R., Gir Idez, F.J.,** Animal performance, carcass traits and meat characteristics of Assaf and Merino × Assaf growing lambs, Livestock Science, 138, 2011, 1, 13-19.
19. **Shirima, E.J.M., Mtenga, L.A., Kimambo, A.E., Laswai, G.H., Mgheni, D.M., Mushi, D.E., Shija, D.S., Safar, J.G.,** Influence of age at entry and level of concentrate feeding on growth and carcass characteristics of feedlot-finished Tanzanian long-fat-tailed sheep, Tropical Animal Health Production, 2014, 46, 815-822.
20. **Sorensen, J.T., Edwards, S., Noordhuizen, J., Gunnarsson, S.,** Animal

production system in the industrialized world, Revue scientifique et technique, 2006, 25, 493-503.
21. **Vlaic, A., Nagy, B., Mireșan, V., Dărăban, S., Coşier, V.,** Experimental Results Obtained in Fattening Norwegian White x Turcana Young Sheep. Bulletin UASMV, Animal Science and Biotechnologies. 2009, 66, 1-2, 226-230.
22. **Vlaic, A., Şichet, C., Oroian, T., Cighi, V., Odagiu, A., Criste, F., Dărăban, S.,** Results concerning the growth dynamics in young sheep Suffolk x Tsigai hybrid and Tsigai from lambing to weaning, Lucrări Ştiințifice Zootehnie şi Biotehnologii, 2008, 41, 2, 799-804.
23. **Voia, S.O.,** Sheep and goats productions – Evaluation tehniques, Ed. Marineasa Timişoara, 2005.

STUDY REGARDING ULTRASOUND AND RADIOGRAPHIC EXAMS RELEVANCE IN URETHRAL LITHIASIS IN DOG

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Summary

The pathology of the urinary tract in dogs take on different aspects, and urolithiasis represents one of the most frequent causes for being presented to the veterinary specialist. Urolithiasis refers to the development of uroliths along the urinary tract, which can be found in the kidneys, urethra, urinary bladder and/or urethra. The aim of this study was to describe the clinical signs and diagnostic imaging of urolithiasis in a group of dogs evaluated in the Faculty of Veterinary Medicine Bucharest. During the study there were examined 28 dogs, using general methods (inspection, palpation) and paraclinical exams (radiographic and ultrasound exams, urinary sediment), obtaining data regarding breed and age for every patient. Urolithiasis is the result of complex disorders at the level of the urinary tract, but also at the level of the whole body. In practice, a complete clinical evaluation is necessary in order to apply an appropriate therapeutic approach.

Keywords: radiography, urolithiasis, ultrasound, dog.

Imaging diagnostic techniques are the most relevant in the case of a condition such as urethral lithiasis, due to their accuracy and high degree of specificity, and also their non-invasive character (9, 16).

Radiological and ultrasound examination are considered to be the golden standard options in the diagnosis of this urinary pathology (1, 17, 20).

Urethral location, with total or partial obstruction, is common in males, due to the location of uroliths before the proximal extremity of the penile bone. Clinically, it might appear: dysuria, strangury and possibly hematuria. The expelled urine is mostly hematuric and contains crystals (14).

The presence of calculi in the urethra is highlighted with particular ease, due to the ecostructure and implicitly their characteristic ultrasound and radiological appearance (5, 8, 19).

Regardless of whether they are radiopaque or radiotransparent, uroliths, from an ultrasound point of view, appear as mobile hyperechoic masses (respecting gravity) that lead to the phenomenon of posterior acoustic shadowing, a cone of shadow that varies depending on the composition and homogeneity of the stone. Blood clots can have different origins and sizes and can be the starting point for the formation of uroliths. Their presence in the free state can create problems of ultrasound diagnosis, compared to calculi (they are accompanied by posterior shadowing) (12, 18).

Materials and methods

The ultrasound was performed with a mini-convex and linear probe with a frequency of 8-10MhZ on Esaote My Lab30 ultrasound machine (Fig. 1). The radiologic examination was performed with DuraDiagnost F30 (Philips China) X-ray machine. The study also included the microscopic examination of our patients' urine, with an Optika optic microscope (Fig. 2), the urine being centrifuged beforehand.

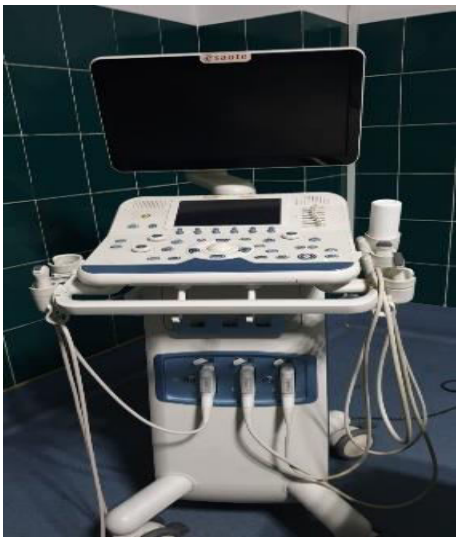

Fig. 1. Ultrasound machine Esaote MyLab30

Fig. 2. Optic microscope Optika

In this study 28 dogs of different breeds and age were included (Table 1). Generally, the animals included in our study, were presented with clinical signs such as: anuria (n=12), strangury (n=10), hematuria (n=6) and excessive grooming in the genital area (6). After the clinical examination was carried out for each of the patients, they were subjected to imaging examinations such as radiography and ultrasound in order to identify possible changes in the urinary system (3, 4, 7, 11, 15, 20).

Table 1

The structure of the group of animals included in our study

Crt. No.	Breed	No. of patients	Sex	Age range
1.	Mixed breed	6	M	6-11 years
2.	Pomeranian	4	M	1-5 years
3.	Teckel	2	M	2-8 years
4.	Bichon Maltese	4	M	1-5 years
5.	Cocker Spaniel	2	M	3-7 years
6.	French Bulldog	3	M	1-6 years
7.	Pekingese	2	M	3-10 years
8.	Shih Tzu	3	M	1-5 years
9.	German Shepherd	2	M	1-8 years

Results and discussions

Among the modifications encountered through the radiographic and ultrasound examination, we observed: ultrasonographically - the presence of corpuscular elements in suspension in the bladder lumen (Fig. 4, Fig. 10) and in the urethral lumen (Fig. 5, Fig. 7, Fig. 9), the presence of the bladder globe (Fig. 3) and radiologically - the presence of millimeter radiopaque structures at the level of the urethral lumen, at the base of the penis (Fig. 6, Fig. 8) (2, 8, 10, 13, 19).

The modifications that were identified through these imaging techniques allowed us to diagnose the patients with urethral lithiasis.

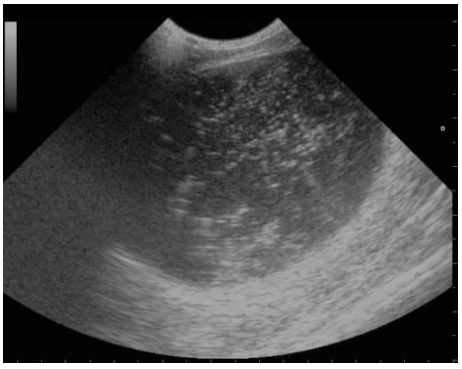

Fig. 3. Cocker spaniel, 4 years. Specific image of bladder globe, with the distention of the parietal structures due to the urethral obstruction

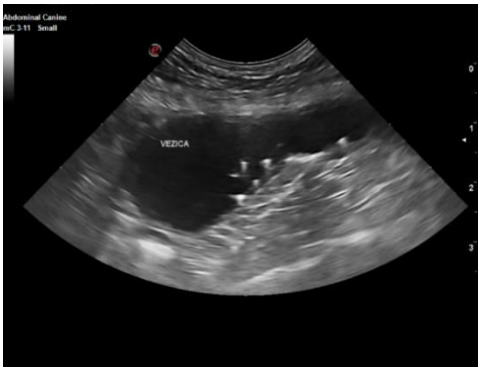

Fig. 4. Mixed breed, 10 years. Urinary bladder with hypoton aspect, the presence of numerous infracentimetric lithiasic elements, moderately parietal reaction

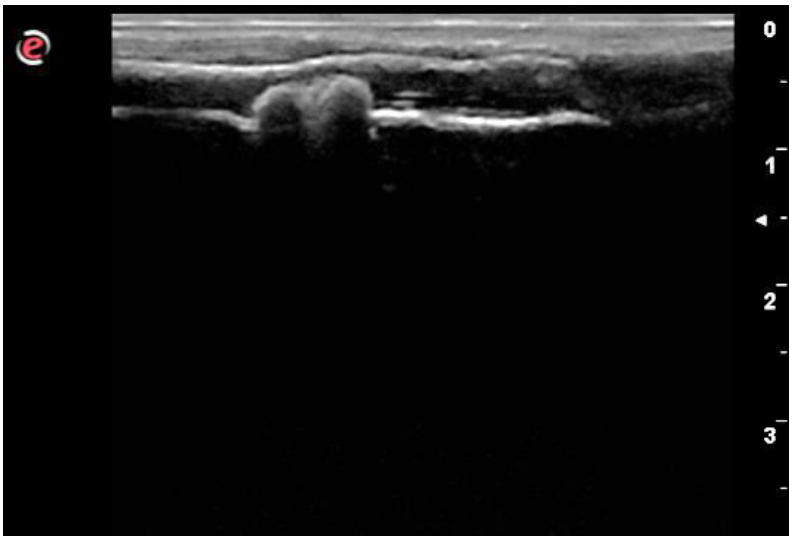

Fig. 5. Shih-Tzu 3 years. The presence of calculi with infracentimetric dimensions, located in the free portion of the urethra, at the level of the penile bone

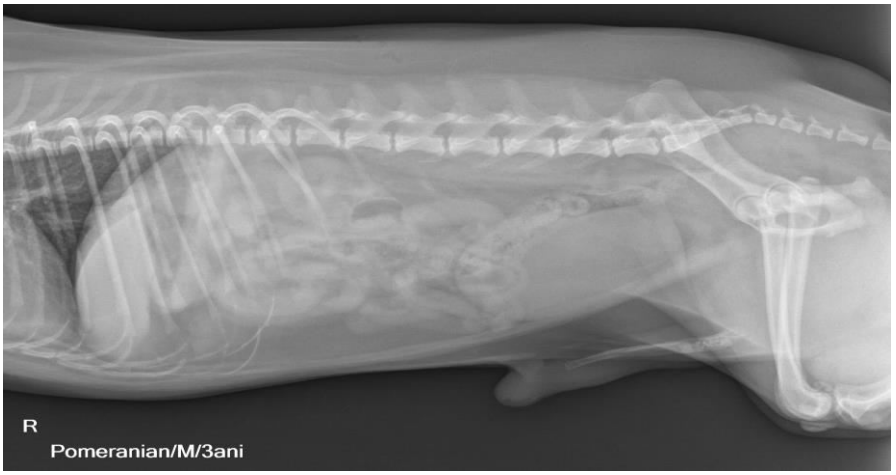

Fig. 6. Urethral lithiasis in a 3-year-old Pomeranian, male, who presented clinical signs as stranguria and hematuria

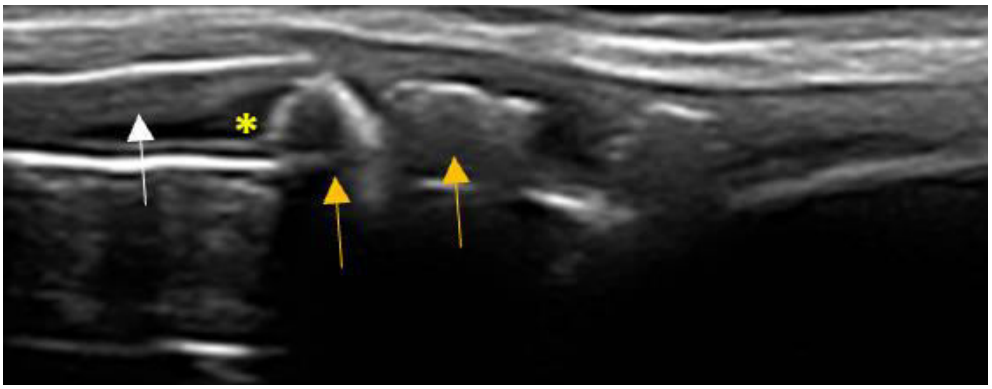

Fig. 7. Teckel, 6 years. The urethra is visible ultrasonographically, the lumen becomes visible (asterisk). It is distended from the anechoic content (due to urethral blockage by stones – yellow arrows). The urethral mucosa is inflamed (reaction of the urethral mucosa – white arrow)

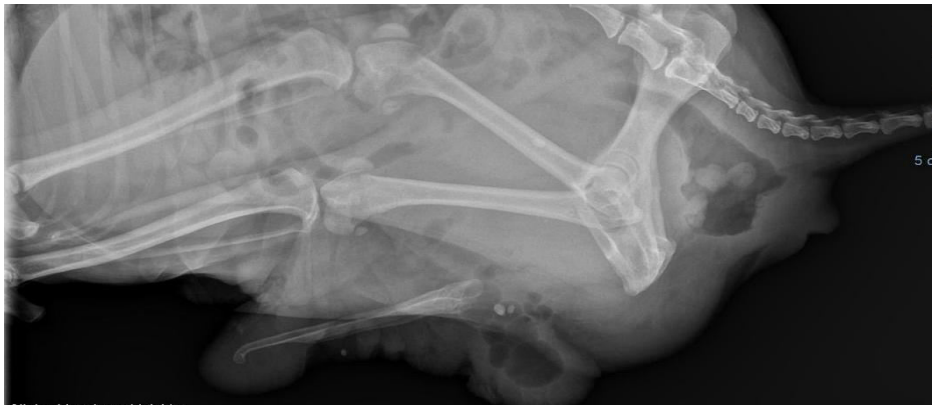

Fig. 8. Mixed breed 6 year male dog, uroliths at the level of the urethra

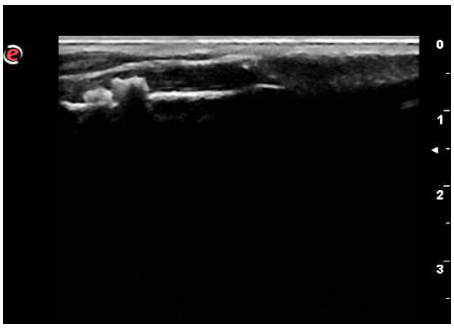

Fig. 9. German Shepherd, 4 years. The presence of two calculi at the base of the penis bone

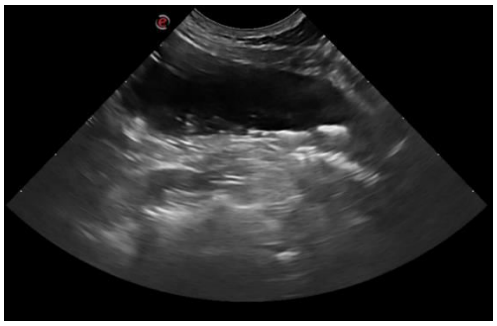

Fig. 10. Pekingese, 5 years. State of semiplenitude, the presence of multiple lithiasic elements of small dimensions

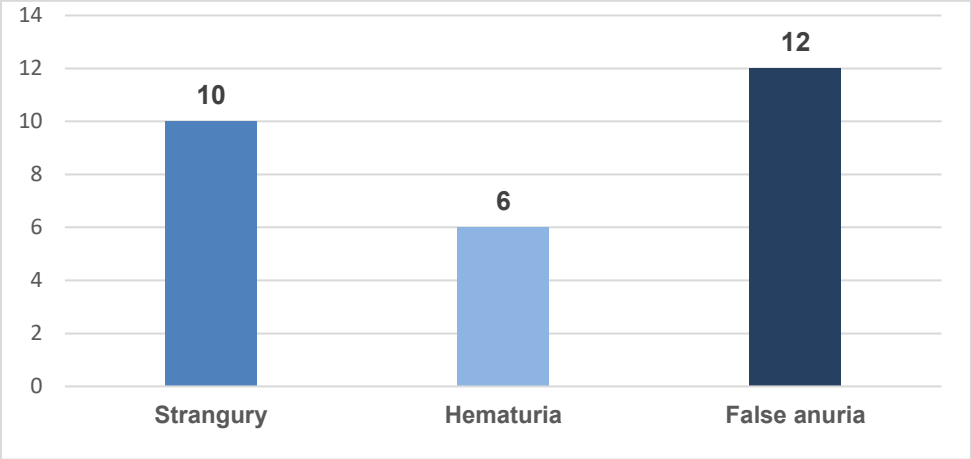

Fig. 11. Graphic representation of the clinic signs of the study group

Regarding the clinical signs of our patients (Fig. 11), false anuria had the highest prevalence (n=12), followed by strangury (n=10) and hematuria (n=6), all of which were a consequence of the presence of uroliths in the urethra, causing an obstructive and irritating effect.

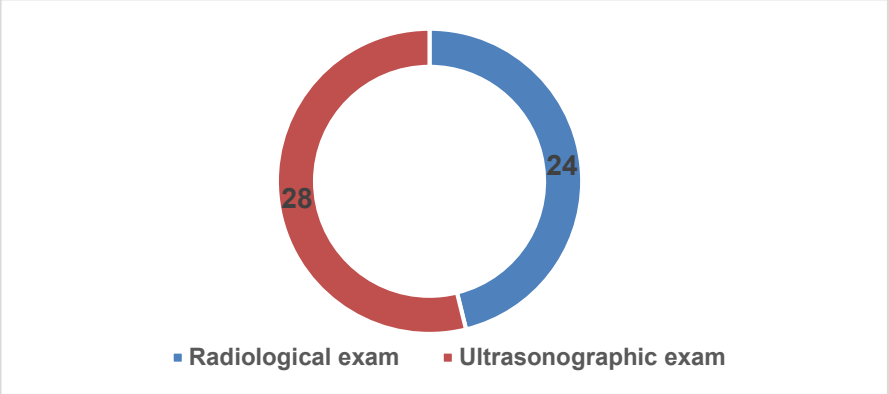

Fig. 12. Graphic representation of the imaging exams performed in our study

The graphic representation of the imaging exams performed is illustrated in Fig. 12, included the radiological exam (n=24) in a percentage of 85.71% and ultrasonographical exam (n=28) in a percentage of 100%, which were used to confirm the suspicion diagnosis of urethral lithiasis in our cases. In our study, the ultrasonographic technique facilitated in confirming more cases than the radiological exam, shows the accuracy and precision of the method of identifying uroliths, which can be radiotransparent or radiopaque, depending on their composition.

Conclusions

The importance of the corroboration of both imaging exams offers and aims to elaborate a better therapeutic approach.

Based on the variety of the clinical signs presented in urethral lithiasis, it is mandatory to approach the cases individually by performing a complete paraclinical examination which includes strip urine, microscopical evaluation of the urinary sediment and common and accessible imaging methods as radiography and ultrasound, leading to a certain diagnosis,

The relevance of the imaging techniques of ultrasound and radiography is indisputable, but when it comes to radiolucent calculi, the only method that can identify their presence is ultrasound.

References

1. **Codreanu, A., Tudor N., Cristian, A.,** Coordonate imagistice în patologia litiazică vezicală la animalele de companie, Practica veterinară, 2002, 3, 34-36.

2. **Codreanu, M.D.,** Clinical-evolutive and ultrasonographic coordinates in urinary bladder diseases in dog, Revista Română de Medicină Veterinară, 2010, 20, 3, 57-70.
3. **Codreanu, M.D., Diaconescu, A.,** Diagnosticul ecografic la animalele de companie, Coral Sanivet, București, 2003.
4. **Codreanu, M.D., Fernoagă, C., Cornilă, C., Codreanu, I., Crînganu, D., Turcitu, M.,** Correlations between the clinical and evolutive expression and type of ultrasound changes in the diagnostic of cavitary organ diseases in dog, Lucrări Ştiinţifice - Medicină Veterinară, Universitatea de Ştiinţe Agricole şi Medicină Veterinară "Ion Ionescu de la Brad" Iaşi, 2010, 53, 3, 612-618.
5. **Codreanu, M.D.,** Patologie şi clinică medicală veterinară, Printech, București, 2020.
6. **Codreanu, M.D.,** Patologie și clinică medicală, Printech, București, 2022.
7. **Codreanu, M.D.,** Puncte critice în abordarea clinică și terapeutică a retenţiei urinare la canide, Vet. Expo., 2012.
8. **Codreanu, M.D.,** Tratat de ultrasonografie veterinară, Medicală, București, 2021.
9. **Crivineanu, M., Codreanu, M.D., Nicorescu, V., Rotaru, E., Papuc, C.,** Studies on the diagnosis and treatment of some urinary bladder disorders in dog, Symposium Contribution of the scientific research to veterinary medicine progress, 2012.
10. **Faria L.A., Meirelles A.É.W.B., Froes, T.R., Cintra, T.C.F., Pereira, D.P., Rodrigues, M.A., Gouvêa, F.N., Pennacchi, C.S., Assaf, N.D., Crivellenti, L.Z.,** Comparison of radiographic methods for detecting radiolucent uroliths in dogs, PLoS One, 2022, 17, 9.
11. **Gary, R.J., Walter, P.A., Feeney, D.A.,** Radiographic and Ultrasonographic Features of Uroliths and Other Urinary Tract Filling Defects, Veterinary Clinics of North America: Small Animal Practice, 1986, 16, 2.
12. **Ling, G.V., Franti, C.E., Ruby, A.L., Johnson, D.L., Thurmond, M.,** Urolithiasis in dogs. I: Mineral prevalence and interrelations of mineral composition, age, and sex, American Journal of Veterinary Research, 1998, 59, 5, 624-629.
13. **Lulich, J.P., Berent, A.C., Adams, L.G., Westropp, J.L., Bartges, J.W., Osborne, C.A.,** ACVIM Small Animal Consensus Recommendations on the Treatment and Prevention of Uroliths in Dogs and Cats, Journal of Veterinary Internal Medicine, 2016, 30, 5, 1564-1574.
14. **Mantis, P.,** Ultrasonography of the Urinary and Genital System of the Dog and Cat, Iranian Journal of Veterinary Surgery, 2008, 63-71.
15. **Maurey, C., Dufayet, C.,** Uro-nephrologie du chien et du chat, Massson, 2005.
16. **Popa (Cristian), A., Iacobescu, M., Codreanu, M.,** Incidence of hematuria in small companion animals, Lucrări Științifice Medicină Veterinară, Timișoara, 2019, LIII, 2, 74-77.

17. **Popa (Cristian), A.M., Șerdean, C., Codreanu, M.,** Oportunitatea ultrasonografiei în afecțiunile vezicii urinare la câine, Simpozionul Ştiinţific Internaţional, Timisoara, 2018.
18. **Nyland, T.G., Mattoon, J.S.,** Small Animal Diagnostic Ultrasound, 2nd Edition, Ed. Saunders Co., 2002.
19. **Robotti, G., Lanfranchi, D.,** Urinary tract disease in dogs: US findings, A mini-pictorial essay, Journal of Ultrasound, 2013, 16, 2, 93-96.
20. **Weichselbaum, R.C., Feeney, D.A., Jessen, C.R., Osborne, C.A., Dreytser, V., Holte, J.,** Urocystolith detection: comparison of survey, contrast radiographic and ultrasonographic techniques in an in vitro bladder phantom, Veterinary Radiology & Ultrasound, 1999, 40, 4, 386-400.

EFFECTS OF ACUPUNCTURE IN PATIENTS SUFFERING OF DEGENERATIVE MYELOPATHY – SYSTEMATIC REVIEW

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Summary

Acupuncture is one of the fifth branches of the Traditional Chinese Veterinary Medicine (TCVM) and it has been used in China since 3000 years ago. It is presumed that in the year 300 BC, it has been used on horses, by people called "Horse priests" at that time. The first official documentation of acupuncture in veterinary medicine was reported in 1971, in United States (USA), when Gene Bruno and John Ottaviano started to use acupuncture for treating horses and small animals. Nowadays, the benefits of acupuncture were forth in medical conditions such as: back pain, neck pain, osteoarthritis, headache, peripheral neuropathy, rheumatoid arthritis, tendinopathy and so on. In the veterinary community, the efficacy of acupuncture is still a controversial subject although the results from human medicine revealed beneficial data. The benefits of acupuncture are presented trough their way of action, by choosing the key points on the patient's body and the insertion of the needles in the muscles stimulates the body to produce his natural healing substances, for example endorphins with the role of pain-relieving. The term "acupuncture" it generally covers a lot of subdivisions of its action, such as laser acupuncture, electroacupuncture, acupressure, all used to treat the pain and stimulate the body. Degenerative myelopathy (DM), is a common neuropathy in old canine patients, and it is found with predilection in German Shepherd dogs. DM affects the spinal cord and has many ways of manifestation, from patients without showing signs of pain, to patients that have neurological deficits of variable severity on the hind legs. Even though acupuncture is not considered the first option of therapy for these patients, there are some studies and data suggesting that using acupuncture or electroacupuncture alongside medical treatment, improved the functional recovery for patients suffering from neurological deficits. However further research and evidence is needed to conclude if acupuncture can be seen as a beneficial post-operative method in the veterinary field.

Keywords: acupuncture, electroacupuncture, German Shepherds, DM.

Acupuncture has its origins from Chinese traditional medicine, and it is used by inserting needles in the patient's body, on very specific points which are known to correspond with the flow of the Qi through the body. Used since the beginning of occidental medicine, its purpose is to ease a lot of health issues (6).

Recently, acupuncture became more known to the public, and it is studied and used also as a treatment for animals.

Over time, the many benefits of acupuncture in animals, focusing on dogs, had been visible. It can help with pain management, respiratory and digestive problems (19). The working mechanism of acupuncture focuses on restoring the energetic balance in the body by stimulating the organism's healing capacity. One of the theories regarding the way acupuncture works is linked to the release of

endorphins, which has a role in easing pain. Also, the stimulation made by the thin needles betters the blood flow, improving the route of the medication and reducing inflammatory processes (1). Acupuncture works best along with physiotherapy and medication (19). There is evidence that acupuncture can have significant effects on the autonomic nervous system, and that electroacupuncture can activate the sympathetic nervous system, which may suppress certain immune responses. Additionally, the adrenal gland and sympathoadrenal medullary axis may be involved in the anti-inflammatory effects of high-frequency acupuncture (3).

Over time, acupuncture developed many branches, such as:

1. Traditional Chinese acupuncture: the most common and used form. Its principle is based on the harmony of yin and yang, along with life energy known as qi (19).
2. Korean acupuncture: its similar to the first one, but it's focused in on-hand techniques and the areas of the upper body (19).
3. Japanese acupuncture: using thinner needles, and it works with massages made with finger pressure along with applying heat on appointed areas (19).
4. Electro-acupuncture: transmission of electrical stimulus through the needles, focusing on treating muscle problems and pain (17).
5. Scalp acupuncture: inserting the needles into the scalp, focusing on neurological problems such as sclerosis or stroke (19).
6. Auricular acupuncture: inserting the needles into ears used to treat chronic pain and anxiety (17).

Degenerative myelopathy it's a disease which targets the spinal cord in dogs. Over time, it can lead to the inability of using hind limbs, caused by the deterioration of the spinal cord (15). The causes are unknown, but it is believed that it's caused by some autoimmune process, which destroys the myelin sheath. Another probability is a genetic disease, a predisposition was observed in certain breeds like German Shepherds, Boxers, and Corgis (5).

Specific symptoms of DM are difficulty standing, incoordination in hind limbs and paralysis. Unfortunately, DM has no cure, treatment being used only to improve the life quality of affected dogs (5, 15).

The goal of treatment in degenerative myelopathy is to improve the quality of life by managing the secondary effects of the disease. Early therapy is important in order to maintain muscle tone, improve neuromuscular function and stimulate the spinal nerves in the affected area. It is also important to regulate the autonomic nervous system to reduce oxidative damage to the degenerating myelinated fibres. Myofascial dysfunction will also be treated in order to release muscle spasms and tension (5, 15).

DM is more likely to occur in the thoracic region of the spinal cord due to the smaller diameter of blood vessels and a lower percentage of radicular artery contributions in this area. This can lead to neural tissue damage from oxidative stress caused by a mutated SOD-1 enzyme. To address this, photo-biomodulation can be

used to improve blood flow to the affected region and other areas with myofascial restrictions. This can enhance the effectiveness of acupuncture therapy (15).

The standard treatment for DM in dogs is:

1. Physiotherapy: used to maintain mobility and avoid muscle atrophy, some of the exercises are stretching and massage (12, 15).
2. Medication: Used to manage the pain, recommended nonsteroidal anti-inflammatory drugs. Vitamins and cholinesterase inhibitors in order to sustain nerve function (12, 15).
3. Assistive devices: wheelchairs and harnesses, to improve their quality of mobility (15).

Looking from the perspective of TCVM (traditional Chinese acupuncture) DM are Wei syndromes in dogs. Patients are Qi and yin deficient (4).

1. Wei Zheng: known as flaccidity syndrome in western medicine, it covers any peripheral nervous system disorder which can cause weakness or numbness. It includes spinal, muscular disorders or sclerosis which manifest itself with paralysis, hemiplegia, or atrophy of the muscles. In this case, the patterns observed are Qi and Yin deficiency and Yin and Yang deficiency (4).
2. Deficiency of Qi: Includes signs such as atrophy of the limbs, listlessness, weak voice, sweating, palpitation, weak pulses, and muscular flaccidity (4).
3. Deficiency of Qi and Yin: mostly in old patients, muscular flaccidity is present again, soreness, weakness of the knees, blurring vision, and red-dry tongue (4).

To define the bigger picture of the benefits of acupuncture, we also made research on the effects of acupuncture in another spinal cord disease, Intervertebral disk disease known as IVDD, in order to state or decline the benefits of acupuncture treatment. Comparative to the DM cases treated with acupuncture, in IVDD, there is a need for many more studies to be made, to see if acupuncture can improve the treatment or even if it works on its own (16).

Intervertebral disc disease (IVDD) is a condition that affects the discs that sit between the vertebrae in the spine. It is commonly seen in dogs, especially those with a long spines, such as dachshunds, beagles, and basset hounds. In IVDD, the discs in the spine can become damaged, which can lead to pain, paralysis, and other complications. Symptoms of IVDD may include difficulty walking, difficulty standing or sitting, using the back legs and changes in behavior. Treatment for IVDD may include medications, physical therapy, acupuncture, or/and surgery, depending on the severity of the condition. It is important to catch IVDD early and provide prompt treatment to improve the chances of good outcomes (10, 16).

Materials and methods

We collected information from many studies and clinical cases where acupuncture or any other form of it was used in patients with degenerative myelopathy, in order to affirm or deny its benefits in their cases.

Before starting to analyze each case in detail we compiled 2 tables (Table 1, Table 2) to show the summary of the most important cases found, with different outcomes to see a clear difference in cases and thus their outcomes.

Table 1

Cases of dogs suffering from degenerative myelopathy and the effects of acupuncture

Dog with quadriplegia	**Positive effects of acupuncture**
Female dog with pregnancy	Positive effects of acupuncture
Dog with paralysis	Euthanasia
Dog with DM	Euthanasia
11 years old Labrador	Positive effects of acupuncture and electroacupuncture
11 years old German Shepherd	Positive effects of acupuncture and electroacupuncture
12 years old Labrador	Positive effects of acupuncture and laser theraphy
9 years old Boxer	Slight improvement

Table 2

Cases of dogs suffering from IVDD and the effects of acupuncture

No. dogs	Age range	Breed	Diagnosis	Effects of acupuncture
1	4 years	Pekinese	IVDD	Improved the patient's mobility, proprioception,and spinal posture
1	12 years	Mixed	IVDD	Improvement in proper walking and flexible head-turning.
12	2-9 years	Poodles, Yorkshire Terriers, Schnauzers, Shi Tzu	IVDD	Recover of 100% of dogs with mild to moderate IVDDm(grade 1-3) , recover of 25% of dogs with severe IVDD (grade 4)
80	1-11 years	Pekinese, Shi Tzu, mixed, Cocker spaniel, etc.	IVDD	Improving ambulation, relieving back pain, and reducing the risk of relapse in paraplegic dogs with normal pain sensitivity.

Results and discussions

In one of the clinical studies that we found, there were 4 cases with degenerative myelopathy, with a clinical symptom of incontinence and the hind limbs were affected. They used electroacupuncture and only in 2 cases they saw improvement. In one case, after 2 sessions of acupuncture, on a dog with quadriplegia, the animal started walking. In another case, which also involved advanced pregnancy, she was suffering from hemiplegia, and after receiving scalp acupuncture, she recovered. In the last described case, they applied on a paralyzed dog acupuncture treatment for 10 weeks. Unfortunately, 2 dogs were euthanized due to the owners' wishes (8).

Another case we found is an 11-year-old Labrador, with a weight of 60 pounds. Being diagnosed with degenerative myelopathy, in her hindquarters, plus arthritis and hip dysplasia. She started being treated with acupuncture and electroacupuncture, to which she reacted well, but after a while, the effectiveness decreased. She started to become weaker. The next step in her treatment was receiving Prolotherapy treatment, and after that, she could walk without crossover. She got 3 prolotherapy treatments and the benefits were visible, improving her balance, and her hind legs staying parallel, but it only lasted for a while.

They couldn't continue the prolotherapy treatment due to her health issues. Unfortunately, the patient had an accident involving her front elbow and needed weeks of recovery. In this period, she received acupuncture and electroacupuncture, and benefits were shown (2).

A German Shepherd female, 11 years old, had asymmetrical paresis of hind limbs, mostly being affected on the left side. She was diagnosed with DM and her treatment was acupuncture, electroacupuncture, and laser therapy to offer analgesia and release muscle tension. Comfort for the patient was shown due to the treatment. Her treatment consisted of CNS points with the role of relaxation. Each session also included effleurage and was closed with skin rolling. Electroacupuncture was introduced only in the third session. She was treated once a week.

Over the treatment, the owner affirmed that she had less discomfort at home, and her anxiety was lowered. The veterinarian affirmed that she maintained her tonus and wiggle her tail for balance (7).

This case focuses on a 12-year-old male, Labrador weighing 40 kg. He was diagnosed with degenerative myelopathy, owner said that he saw slight changes in his dog's gait, starting about 8 months prior veterinary visit, difficulty jumping and his hind limbs being weak. He also said that the dog couldn't always keep his posture. The treatment used was fish oil, Meloxicam and Tramadol the main focus being managing the pain and inflammation. He also received therapeutic massage, acupuncture, spinal therapy, and laser therapy in order to maintain neuromuscular function. After one year of therapy, the dog could walk 20-30 minutes without problems (14).

A 9-year-old male Boxer was diagnosed with degenerative myelopathy, being gene tested which confirmed a mutation of the SOD1 gene. He had ataxia during gait on both hind limbs, and on the left hind limb paw placement was absent. His treatment consisted of hydrotherapy or physiotherapy twice a week, electrotherapy, and exercise at home. This lasted for 17 sessions. The response to the treatment is considered positive, gaining improvement in the gait and paw placement and improvement in his posture. After a while, the symptoms got worse, and the last solution was the use of wheels for his hind limbs while still continuing IVDD cases treated with acupuncture (6).

A Pekingese dog was taken to a veterinary medical center due to severe hind limb problems including ataxia, atrophy, and paresis. The diagnosis, based on physical examination, neurological assessment, and /or CT scan, revealed multiple cases of intervertebral disc disease in the thoracic and cranial lumbar spine. The traditional veterinary medicine diagnosis was kidney Yang deficiency syndrome. After initial treatment with high-dose prednisolone did not show improvement, the dog was treated with electroacupuncture and herbal medicine for 6 months which significantly improved the patient's mobility, proprioception, and spinal posture.

A non-ambulatory, castrated male dog with tetraplegia was diagnosed with spinal stenosis at the C3-C4 level. According to traditional Chinese veterinary medicine, the dog had local Qi and blood stagnation, spleen Qi deficiency, blood deficiency and kidney Yang deficiency. The dog was treated with a combination of acupuncture, Chinese medicine iontophoresis and laser therapy. After 12 treatments, the dog showed significant improvement in ambulation function, including proper walking and flexible head-turning (13).

In this study, 12 dogs with intervertebral disc disease (IVDD) were treated with acupuncture. The ages of the dogs ranged from 2 to 9 years old, with an average age of 3.6 years, and the affected breeds included miniature Poodle, Yorkshire Terrier, Miniature Schnauzer, Shih Tzu, and mixed breeds. Symptoms of IVDD included spinal pain (hyperesthesia) at certain levels of the thoracolumbar spine and radiographic evidence of narrowed intervertebral disk space, osteophyte formation, or mineralized intervertebral disk in severe cases. Acupuncture treatment consisted of several steps including the use of specific points on the body and targeting certain areas of the spine. The dogs received 2-3 acupuncture sessions per week, and the duration of treatment varied from 5 days to 6 weeks for mild to moderate cases and 7 weeks for severe cases. Overall, 100% of dogs with mild to moderate IVDD (grades I to III) recovered after 1-12 treatments, while 25% of dogs with severe IVDD (grade IV) recovered after 15 treatments (9).

This study investigated the effectiveness of combining electroacupuncture and acupuncture with medication in the treatment of thoracolumbar intervertebral disc herniation in paraplegic dogs with normal pain sensitivity. The medical records of 80 dogs were reviewed and divided into two groups: one group received only conventional medicine(prednisone) (n=37) and the other group received both conventional medicine and electroacupuncture and acupuncture(n=43).

Electroacupuncture was applied at specific points on the body and acupuncture was performed at certain points near the affected area and on the opposite side of the body. The treatment's success was evaluated based on post-surgery neurologic function, ambulation, relapse rate, complications, and urinary function. The results showed that the group that received both conventional medicine and electroacupuncture and acupuncture had a higher rate of ambulation recovery and shorter recovery time for ambulation and back pain relief compared to the group that only received conventional medicine. The group that received both treatments also had a lower rate of relapse. These findings suggest that the combination of electroacupuncture and acupuncture with conventional medicine is more effective than conventional medicine alone in improving ambulation, relieving back pain, and reducing the risk of relapse in paraplegic dogs with normal pain sensitivity (9).

Comparative to the DM cases treated with acupuncture, in IVDD are many studies to see if acupuncture can improve the treatment or even if it works on its own (Fig. 1, Fig. 2).

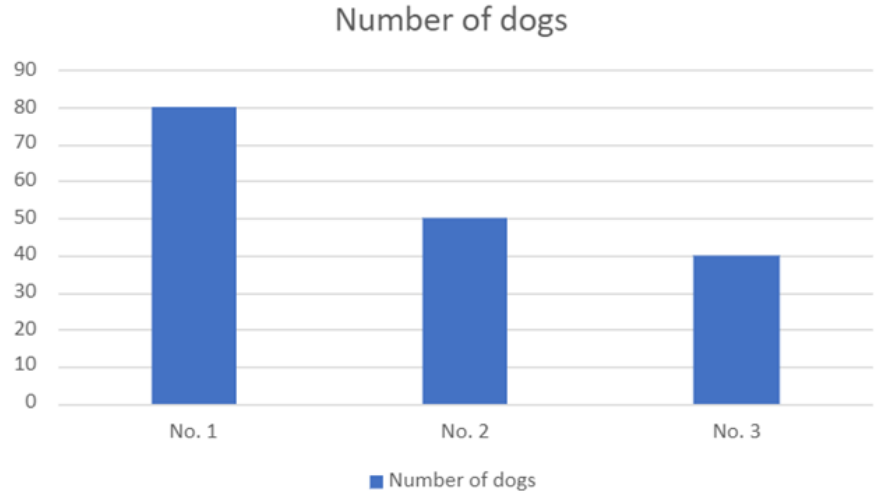

Fig. 1. Number of dogs treated with acupuncture in IVDD

Treatment more effective	Reducing the time of recovery by half	Treatment more effective
P = 0.015	P = 0.015	P = 0.05
+	+	+
Prednisone	Unknown	Hemilaminectomy
IVDD,paraplegia	IVDD	IVDD
80	50	40
No. 1	No. 2	No. 3

Fig. 2. Treatment results of dogs paired in groups

Conclusions

Based on the information provided, it appears that the effectiveness of acupuncture in the treatment of degenerative myelopathy varies from case to case. In some cases, acupuncture or electroacupuncture seemed to offer some improvement, while in others the benefits were short-lived or not observed. It is also worth noting that other forms of treatment, such as hydrotherapy, physiotherapy, therapeutic massage, spinal therapy, laser therapy and the use of wheels, were also used in some of the cases and may have contributed to the overall treatment outcome.

In summary, acupuncture and electroacupuncture had been shown to be effective in improving mobility and relieving pain in dogs with intervertebral disc disease (IVDD). These treatments have been used alone or in combination with other therapies, such as herbal medicine, iontophoresis, and laser therapy. The duration of treatment and the degree of improvement varied among the cases, but overall, acupuncture and electroacupuncture appear to be a promising treatment options for dogs with IVDD. It should be noted that these results come from a limited number of cases and further research is needed to fully understand the effectiveness of acupuncture in these conditions.

As we can see, the collected data of acupuncture used in another spinal disease, IVDD, is much more needed. The goal was to show the lack of studies made on the effects of acupuncture in degenerative myelopathy compared to other spinal diseases. Starting from this idea, it is hard to define a clear point if acupuncture is beneficial or not in degenerative myelopathy, due to insufficient information and clinical studies.

References

1. **Aldridge, C., Behrend, E.N., Kemppainen, R.J., Lee-Fowler, T.M., Martin, L.G., Ward, C.R., Lee, H.P.,** Comparison of 2 Doses for ACTH Stimulation Testing in Dogs Suspected of or Treated for Hyperadrenocorticism, Journal of Veterinary Internal Medicine, 2016, 30, 5, 1637-1641.
2. **Babette, G.,** Practical Application of Prolotherapy in Canines: Case Studies, Journal of Prolotherapy, 2009, 1, 3, 179-180.
3. **Baker-Meuten, A., Wendland, T., Shamir, S.K., Hess, A.M., Duerr, F.M.,** Evaluation of acupuncture for the treatment of pain associated with naturally-occurring osteoarthritis in dogs: a prospective, randomized, placebo-controlled, blinded clinical trial, BMC Veterinary Research, 2020, 16, 1.
4. **Clemmons, R.M.,** TCVM Treatment of Degenerative Myelopathy, SACS/College of Veterinary Medicine, University of Florida, 2013.
5. **Cohen, A., Dockweiler, J.,** Degenerative Myelopathy, Cornell University, College of Veterinary Medicine, 2020.
6. **Corral, C.,** Canine Degenerative myelopathy, The Veterinary Nurse, 2020.
7. **Curacore Vet,** Degenerative Myelopathy in an 11 year old German Shepherd, 2019.
8. **Gulanber, E.R.,** The clinical Effectiveness and Application of Veterinary Acupuncture, G&G Academia, American Journal of Traditional Chinese Veterinary Medicine, 2008, 3, 9-22.
9. **Han, H.-J., Yoon, H.-Y., Kim, J.-Y., Jang, H.-Y., Lee, B., Choi, S. H., Jeong, S.-W.,** Clinical Effect of Additional Electroacupuncture on Thoracolumbar Intervertebral Disc Herniation in 80 Paraplegic Dogs, American Journal of Chinese Medicine, 2010, 38, 6, 1015-1025.
10. **American College of Veterinary Surgeons**, Intervertebral Disc Disease.
11. **Jeong, J.H., Song, J.Y., Jo, H.G., Kim, J.M., Yoon, S.S., Park, C., Kim, H.Y.**, Simple Acupoints Prescription Flow Chart Based on Meridian Theory: A Retrospective Study in 102 Dogs, Evidence-Based Complementary and Alternative Medicine, 2013, 1-13.
12. **Kathmann, I., Cizinauskas, S., Doherr, M.G., Steffen, F., Jaggyet, A.,** Daily Controlled Physiotherapy Increases Survival Time in Dogs with Suspected Degenerative Myelopathy, Journal of Veterinary Internal Medicine, 2006, 20, 4, 927-932.
13. **Kim, S.H., Kim, S.N., Lee, K.C., Kim, M.S.,** Treatment of Multiple Thoracolumbar Intervertebral Disc Disease using Electro-acupuncture and Oriental Herbal Medicine in a Dog, Pakistan Veterinary Journal, 2012, 32, 4, 631-634.
14. **LoGiudice, R.J., Starr, L.,** Rehabilitation for Geriatric Patients, Canine Sports Medicine and Rehabilitation, 2018, 454-478.
15. **White, A.,** A brief history of acupuncture, Heberden Historical, Rheumatology, 2004, 43, 662- 663.

16. **Xie, H., Preast, V.,** Xie's Veterinary acupuncture, Ames, 2007, Blackwell Publishing, Oxford, UK.
17. **Xie, Z.F., Dong, H.,** Acupuncture: review and analysis of controlled clinical trials, World Health Organization, 2002.
18. *** **Lundgren, B.,** Degenerative myelopathy leads to paralysis of Dog's Hindquarters, Veterinary Partner, 2007, https://veterinarypartner.vin.com/default.aspx?pid=19239&id=4952659
19. *** **Morrison, B.J.,** IVDD (Intervertebral Disc Disease) in dogs, PetMD, 2022, https://www.petmd.com/dog/conditions/neurological/c_dg_intervertebral_disc_disease
20. *** **Murray, P., Shurtleff, D., Langevin, H.M.**, Acupuncture: What you need to know, National Center for Complementary and Integrative Health, 2022, https://www.nccih.nih.gov/health/acupuncture-what-you-need-to-know

A COMPARATIVE STUDY REGARDING THE MICROSCOPIC AND CULTURAL MORPHOLOGY OF *BACILLUS ANTHRACIS, BACILLUS CEREUS* AND *BACILLUS MYCOIDES* SPECIES

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Summary

Bacillus anthracis, *Bacillus cereus* and *Bacillus mycoides* species show many biological similarities, especially of microscopic morphology and cultivability (2, 3, 4, 9). They belong to the genus *Bacillus*, family *Bacillaceae*, along with numerous other species (17). Contrary to the mentioned similarities, the high pathogenicity factors are found only in the *Bacillus anthracis* species. Because identification of the morpho-cultural pathogenic species can be confusing, it must be well evaluated. For this purpose, we analyzed the characteristics of the three microbes in the usual culture medium, but also in liver and blood (plasma separately as well, experimentally inoculated). We used the vaccine strain of *Bacillus anthracis* 1190-R (prof. Nicolae Stamatin) and two laboratory strains: *Bacillus cereus* and *Bacillus mycoides*. We followed the shape of bacteria, the size and appearance of their extremities, the association of bacteria in their own characteristics, the formation of species and their positioning and the capsulogenesis. We also noted down the characters of the pure cultures. The information we used from external sources can be found in the bibliographical reference.
Keywords: *B. anthracis*, *B. cereus*, *B. mycoides*, characteristics.

The aim of this study consists in the evaluation of few phenotype similarities between a pathogenic bacterium species - *Bacillus anthracis*, and other two related species - *Bacillus cereus* and *Bacillus mycoides*. All three species belong in the Bacillaceae family, and are sporogenous bacteria, usually present in the soil (5, 12, 17, 18). From the contaminated soil, they can easily enter into animal or human organisms, and contaminate the intestinal lumen. In the case of pathogenic species *Bacillus anthracis*, the infection - ANTHRAX - is very dangerous and, more often, rapidly fatal, for many animals, special mammals, and humans, too (6, 7, 8).

Because the intestinal flora invades in short time majority of organs or corps tissues, it is important to differentiate in good conditions between the real pathogen and different similar microbes, simultaneous present into pathological samples. In addition, it is necessary to isolate *Bacillus anthracis* from the soil samples in case of suspected/possible contamination of some geographic areas, or in case of the destruction or absence of the animal corps, when the Antrax's suspicious exists (13, 14, 16).

In addition, sometimes it is necessary to differentiate between the three bacteria in case of food intoxication, when the aliments - e.g. meat, rice, milk, are examined to detect the pathogen (*Bacillus anthracis*, rarely *Bacillus cereus*).

Bacillus anthracis, *Bacillus cereus* and *Bacillus mycoides* are Gram positive bacteria, present into infected corps (14). The pathogenic high properties of *Bacillus anthracis* are responsible for Anthrax, a very dangerous zoonotic infection (some sources claim that zoonosis was also used as a biological weapon in the First World War, but also at the beginning of the 21st century in the USA) (11, 24).

The pathogenic character of *Bacillus anthracis* is correlated with endospore bacterial forms because they can invade very easily the organism from the contaminated environment (18, 23). The bacterial spores are rapidly included into macrophages of infected hosts, but some immune cells are destroyed by lysis phenomenon, and the consequence is the spread of the pathogen in different organic areas. In case of respiratory infection, the infected macrophages are transferred to mediastinal lymph nodes. Into macrophages, *Bacillus anthracis* spores survive a long time, even 60 days, and the corresponding incubation period of zoonoze can vary between two and 6-8 weeks, for contamination by inhalatory way (23). The contrary, this latent period usually does not exist in cutaneous Anthrax infection (*Pustula maligna*).

Bacillus cereus is usually found in soil and water, but it also can be found in products that are but can also be found in products that come from animals that have been in contact with infected soil, such as milk and milk derived products (1). It can persist and survive in harsh environmental conditions by the production of endospores and formation of biofilms. The spores of *Bacillus cereus* are elongated, characterized by a core surrounded by an inner membrane, peptidoglycan cortex, inner coat and outer coat. The bacterial spores have no metabolic activity and are resistant to heating, freezing, drying, and gamma-ray and ultraviolet radiation These spores are extremely resistant to environmental assaults that would normally kill vegetative bacteria, thereby facilitating persistence in the environment until more favorable conditions return (19, 20, 21).

Even if the pathogenicity of the *Bacillus mycoides* is not as high and to be taken into account as that of other bacilli, its beta hemolytic property and specialized studies have shown that under favorable conditions, it can end up infecting the blood of a human host (22, 25).

The bacteriologic analysis for determine the cause of Antrax suppose the identification of *Bacillus anthracis* in different biological samples, like spleen, liver, unclothed blood, bone marrow, exsudates, intestinal contents, also in soil samples. In corps and soil samples, normally are founded anthracoides bacilli, like *Bacillus cereus*, *Bacillus mycoides* or other, that decompose the tissues and present an hemolytic properties (15).

In these general conditions, it must be obtained a very high differentiation between the pathogen and other similar, but non-pathogenic bacteria, to avoid an erroneous diagnostic.

Materials and methods

For analyze and compare the phenotype similarities and the differences between *Bacillus anthracis* and biologic related bacteria, we selected a non-pathogenic strain of *Bacillus anthracis* (more exactly the vaccin alive strain 1190-R, used for immunization of domestic animals, realized by Prof. Nicolae STAMATIN, 1936), and two anthracoids: *Bacillus cereus* and *Bacillus mycoides*. The origin of all bacterial strains is the Microbiology Discipline's bacterial collection of the Faculty of Veterinary Medicine, Bucharest.

We used fresh pure bacterial cultures, few culture media (nutrient broth, nutrient agar, sheep blood agar, cow blood agar), also some biologic products: fresh blood unclothed, also clothed, obtained from health sheep and cows, and fresh cow's liver to obtain a better analysis of the three bacteria (natural culture environments being good supporters for the development of bacteria) (2).

We also used bright field and dark field microscopes, aerobic incubator, magnifying glass, centrifuge, slides, insemination loops, and colorant reagents for Gram stained method.

In the first step, we regenerated the bacterial spores from the didactic microbiological collection (old and dry nutrient agar slants) by their inoculation into nutrient broth and nutrient agar (test tubes and Petri dishes), and cultivate in a normal atmosphere, at 37°C, for 24 hours.

In the second time, we prepared and inoculated the blood agar in Petri dishes - separately sheep blood agar and cow blood agar, for evaluate the hemolytic activity of the studied bacteria (incubation in a normal atmosphere, 37°C) (Fig. 1)

We analyzed the cultural aspects - macroscopic examination - in liquid and solid culture media, after 24 hours of incubation, also during a few days, at room temperature. At the same time, we were focused by bacterioscopic examination, bacterial smears colored using Gram stained method, also the fresh uncolored samples, between slide and lamella, for dark field microscopy.

Simultaneously, we inoculated pure bacterial spore suspension in saline solution (NaCl 9‰) into fresh heparinized blood, in plasma blood samples, also in serum blood samples, and into fresh cow's liver. All these nutritive supports were incubated in normal atmosphere, at 37°C, for 24 hours.

Fig. 1. Few aspects during bacteriological analysis

We appreciated, immediately after incubation period, and after that, for few days, at room temperature, and normal atmosphere the next bacterial characteristics, for each strain:

- The aspect of nutrient broth culture
- The aspect of the colonies on nutrient agar and blood agar
- The motility test (in fresh preparations)
- The capacity to develop in biologic samples (integral blood, serum blood, plasma blood, liver
- The bacterial morphology after bacteria inoculation into biologic samples

We noted and analyzed all information from our study finally we established a few general conclusions. All images are original and conquer to an eloquent interpretation of the data.

Results and discussions

For the regeneration of bacterial spore stage, we observed no differentiation between *Bacillus anthracis* 1190-R, *Bacillus cereus* and *Bacillus mycoides*; all strains developed rapidly and abundant in nutrient broth, also on nutrient agar (test tubes and Petri dishes).

In inoculated culture media, after incubation, the macroscopic and microscopic observations showed a pronounced development of vegetative cells of

Bacillus cereus and *Bacillus mycoides*, but *Bacillus anthracis* having only a strong presence of spores, not the vegetative forms.

Thus, we confirmed the fact that these two bacterial strains have a medium development at room temperature, *Bacillus cereus* being able to develop at temperatures above 10°C, and *Bacillus mycoides* at temperatures above 10-15°C. The data were most prominently observed on blood agar. We also noticed a certain affinity of these bacilli for sheep and cow blood. Thus, *Bacillus anthracis* preferred cow blood, *Bacillus cereus* sheep blood, and *Bacillus mycoides* had about the same stage of development on both types of blood.

For culture obtained at room temperature, we observed the microscopic aspects, presented in the next table (Table 1).

Table 1

Information on bacteria aspects and cultures at room temperature

Culture media	***Bacillus anthracis* 1190-R**	***Bacillus cereus***	***Bacillus mycoides***
Nutrient agar	Large bacterial population; vegetative cells, no spores	Well developed; spores	Well developed
Nutrient broth	Well developed; longer chains of vegetative bacilli	Rare chains of bacilli; confirmation of the presence of bacterial spores	Well developed
Sheep blood agar	Weaker development than in the case of the sample with cow's blood	Developed more abundantly for the sample with sheep blood	Development at an early stage
Nutrient broth enriched with sheep blood	The appearance of bacterial chains is confirmed	Long bacterial chains	Well developed
Cow blood agar	We noticed a more abundant development than on sheep's blood	Weaker development than in the case of the sample with sheep blood	Development at an early stage
Nutrient broth enriched with cow blood	More numerous chains, better development than for the sample with sheep blood	Shorter chains of bacilli	Well developed, better than for the sheep blood sample
Plasma sheep blood	The bacteria presence was not detected	The bacteria presence was not detected	The bacteria presence was not detected

Plasma cow blood	The bacteria presence was not detected	The bacteria presence was not detected	No bacilli were observed, but some fragments can be seen (possibly remnants from the inoculation)
Sheep serum blood	The bacteria presence was not detected	The bacteria presence was not detected	The bacteria presence was not detected
Cow serum blood	The existence of a single chain was observed in the microscopic field	The bacteria presence was not detected	The bacteria presence was not detected
Fresh unclothed sheep blood	The bacteria presence was not detected	The bacteria presence was not detected	Chains begin to form
Fresh unclothed cow blood	The bacteria presence was not detected	The bacteria presence was not detected	More abundant development, more numerous chains

About the approximate incapacity of bacteria to develop in good conditions in integral (non-diluted) biological products - in our experiment: fresh unclothed blood, serum blood or plasma blood, a possible explanation consists in the immunological properties of these raw products (non thermic treatment) (10, 11). However, at room temperature, few bacterial cells were observed under the microscope, perhaps the temperature inhibited the vegetative transformation of the spores.

Between *B. anthracis* 1190-R, *B. cereus* and *B. mycoides* strains, the differentiation on their capacity to develop at room temperature are not very evident. We consider that *Bacillus anthracis* is partially affected, while *Bacillus cereus* and *B. mycoides* are neutralized by blood/plasma.

To evaluate these biological aspects, we hope to continue the study in another session.

Our observations regarding biologic behavior of the three *Bacillus* strains in cultural conditions similar to the natural ones (level of temperature, time of incubation) are based on many data obtained by inoculation of bacterial pure spores suspensions in different nutritive substrates, and incubation at 37°C for 24 hours, in normal atmosphere. The important results are presented in the next table (Table 2).

Table 2

Information on bacteria microscopic aspects and cultures obtained by 37℃ incubation

Culture media	***Bacillus anthracis* 1190-R**	***Bacillus cereus***	***Bacillus mycoides***
Nutrient agar	*Microscopic*: Medium size bacilli, Gram +, short chains, isolated; central ovoid spores (Fig. 12)	*Microscopic*: large, robust bacilli, Gram +, isolated and short chains bacteria (Fig. 14)	*Microscopic*: Large, linked bacilli, "knotted" appearance (Fig. 15)
	Macroscopic: large non pigmented colonies, Rough type (Fig. 2)	*Macroscopic:* large, non pigmented colonies, opaques, Rough type, larger than B. anthracis colonies (Fig. 3)	*Macroscopic:* large Rough type colony, occupied the entire surface of the Petri dish (Fig. 4, 5)
Sheep blood agar/cow blood agar	*Microscopic* Medium and large chains of Gram + bacilli, many spores in chains, intens developed in cow blood agar than sheep blood agar	*Microscopic* *Cow blood sample:* medium and large chains of Gram + bacilli, some cells present the ovoid central or sub-central spores *Sheep blood sample:* larger Gram + bacilli, short chains, few ovoid and not evident deformed spores	*Microscopic* *Cow blood sample:* very long chains of coccobacilli or short Gram + bacilli, vegetative forms *Sheep blood sample:* better developed, very long chains of Gram + bacteria (knotted or tangled appearance); vegetative forms
	Macroscopic Non hemolytic bacteria culture (Fig. 10)	*Macroscopic* Complete and large hemolysis (Fig. 8, 9)	*Macroscopic* Complete and narrow hemolysis, stronger for sheep blood agar than for cow blood agar (Fig. 11)
Nutrient broth enriched with blood	*Microscopic* More developed and longer bacilli chains, Gram +, vegetative forms in sheep blood nutrient broth	*Microscopic* In the cow blood nutrient broth we detected an abundant presence of Gram +, vegetative bacilli, isolated, diplo and short to medium chains	*Microscopic* Abundant bacteria cells, very long chains, more evident in the cow's blood nutrient broth
	Macroscopic Clear appearance, blood cells were deposed at the	*Macroscopic* Abundant floating material, a medium	*Macroscopic* Strong hemolysis in both samples (sheep and cow

	bottom of the test tube (non-hemolysis); A little to medium bacterial gray sediment	turbidity (for the sheep blood sample); The complete hemolysis caused red color of the medium; A medium size bacteria sediment (in the cow blood nutrient broth culture)	blood), more abundant and larger filaments in the sheep blood sample
Plasma blood	*Microscopic* Undetectable culture	*Microscopic* Undetectable culture	*Microscopic* Undetectable culture
	Macroscopic Undetectable culture	*Macroscopic* Small sediment, more abundant in cow, no turbidity	*Macroscopic* A small sediment
Serum blood	*Microscopic* Medium Gram + bacilli, better development on cow's blood	*Microscopic* Undetectable culture	*Microscopic* Cow sample - long chains of Gram + bacteria Sheep sample - just few bacteria remains visible
	Macroscopic No ring appears, clear appearance, red blood sedimented cells (non hemolysis)	*Macroscopic* Hemolytic activity (stronger in cow sample); the bacterial sediment presence	*Macroscopic* Strong hemolysis; The sediment in the cow blood
Fresh unclothed blood	*Microscopic* Small number of Gram + bacilli (Fig. 13)	*Microscopic* Undetectable culture	*Microscopic* Undetectable culture
	Macroscopic No ring, a medium turbidity, cow's blood shows a small degree of hemolysis (Fig. 6)	*Macroscopic* Cow-slightly hemolyzed red blood cells (Fig. 7)	*Macroscopic* Slightly hemolyzed red blood cow’s cells
Cow liver	*Microscopic* A good development of the Gram + bacilli, isolated and short chains; many bacteria are sporulated, with a central ovoid non deformed spores	*Microscopic* Well developed, small and medium chains of robust Gram positive bacilli (similar to B. anthracis)	*Microscopic* Much more abundant, medium chains of Gram + bacilli (Fig. 16)

The liver samples presented a bad smell, and an early stage of putrefaction is observed. We also identified the existence of some yeast in early stages of development.

Following the mobility test performed on all three bacilli, the results showed that only *Bacillus cereus* is a mobile bacterium.

In the next original images, we presented some conclusive aspects about macroscopic pure culture examination.

Fig. 2. *Bacillus anthracis* on nutrient agar

Fig. 3. *Bacillus cereus* on nutrient agar

Fig. 4. *Bacillus mycoides* on nutrient agar

Fig. 5. *B. mycoides* on usual culture media

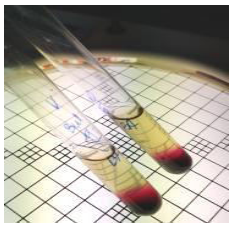

Fig. 6. *B. anthracis* on nutrient broth enriched with cow blood

Fig. 7. *B. cereus* on nutrient broth enriched with sheep blood

Fig. 8. *B. cereus* on sheep blood agar

Fig. 9. *B cereus* on cow blood agar

Fig. 10. *B anthracis* on sheep blood agar

Fig. 11. *B mycoides* on sheep blood agar

In the next original images, we presented a few aspects, conclusive for microscopy examination, on Gram stained smears, realized on pure culture and biologic products, too.

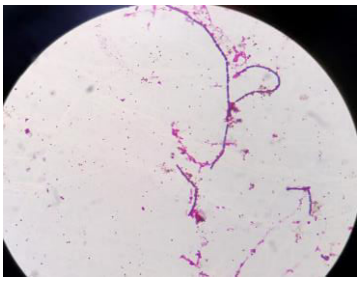

Fig. 12. *B. athracis* on nutrient agar

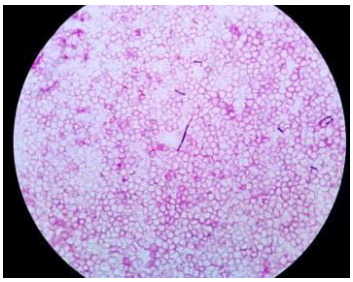

Fig. 13. *B. anthracis* in sheep blood

Fig. 14. *B. cereus* on nutrient agar

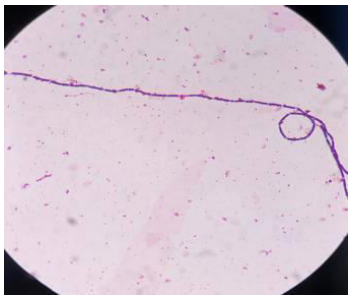

Fig. 15. *B. mycoides* on on nutrient agar

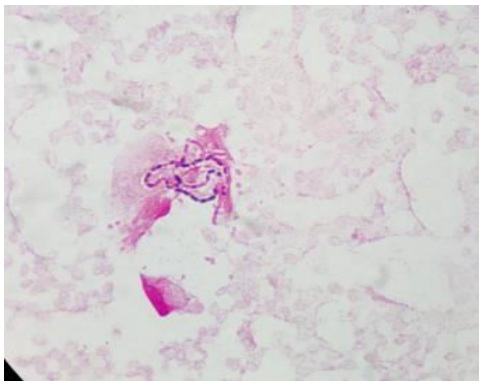

Fig. 16. *B mycoides* on fresh unclothed cow blood

Conclusions

This microbiological study and his results lead us to present a few general conclusions.

About the microscopic morphology, examined in usual culture media - nutrient broth and nutrient agar, exist the possibility to differentiate between *Bacillus anthracis* 1190-R, *Bacillus cereus* and *Bacillus mycoides*, especially on solid media.

About the macroscopic aspects, we established few similarities between *Bacillus anthracis* 1190-R and *Bacillus cereus*, especially on solid media. All three strains produced large rough type colonies, but *Bacillus mycoides* colony was immense.

Bacillus mycoides present a characteristic culture aspect on nutrient broth, and this is very similar with pathogenic *Bacillus anthracis* strains, and different from *Bacillus anthracis* 1190-R, the vaccinal strain.

The possibility to cultivate all three *Bacillus* strains into biological products - sheep and cow fresh blood, serum blood and plasma blood, at room temperature and 37℃, indicate, generally, an impossibility to obtain culture and to examine the biologic properties of bacteria.

About the hemolytic activity (analyzed on solid culture media and liquid culture media), *B. cereus* strain induces a complete hemolysis (on red sheep and cow blood cells), also *B. mycoides* (but not evident on cow blood agar). *B. anthracis* does not induce an evident hemolysis.

In cow liver inoculated separately with the three analyzed strains, the development of bacteria was evident, with few differentiation.

About the motility test, *Bacillus anthracis* and *Bacillus mycoides* are not mobile bacteria.

Acknowledgement

Lecturer DVM Ph. D B.A.TAȘBAC, for all the help given in writing this paper, the guidance, teaching, trust and his availability for us, also for the materials made available and the study resources.

At the same time, we thank him for the counselling in the secrets of microbiology since the first laboratory of practical works in the faculty.

References

1. **Abdul-Hadi, A.H, Abdul A'al, I.N., Abdul-AlRazak, O.,** Morphological and microscopical comparison features of Bacillus cereus isolates, Kufa Journal for Veterinary Medical Sciences, 2011, 2, 2.
2. **Buiuc, D., Neguţ, M.,** Tratat de Microbiologie clinică, Ed. a III-a, Editura Medicală, Bucuresti, 2017.
3. **Dimache, Gh., Panaitescu, D.,** Microbiologie şi parazitologie veterinară, Ed. Uranus, Bucureşti, 1994.
4. **Fiţ, N.I.,** Laboratory manual Special Microbiology, Editura AcademicPres, Cluj-Napoca, 2016.
5. **Moga Mânzat, R.,** Boli infecţioase ale animalelor. Bacterioze, Editura Brumar, Timişoara, 2001.
6. **Perianu, T.,** Boli infecţioase ale animalelor. Bacterioze, Editura Fundaţiei „Chemarea", Iaşi, 1996.
7. **Perianu, T.,** Tratat de boli infecţioase ale animalelor. Bacterioze, Editura Universitas XXI, Iaşi, 2011.
8. **Răducănescu, H., Bica-Popii, V.,** Bacteriologie veterinară, Ed. Ceres, Bucureşti, 1986.
9. **Răpuntean, Gh., Răpuntean, S.,** Bacteriologie veterinară specială, Editura AcademicPres, Cluj-Napoca, 2005.

10. **Realpe, M.E., Hernández, C.A., Agudelo, C.I.,** Especies del género Bacillus: morfología macroscópica y microscópica, Imagenes en Biomedicina, 2002, 22, 2, 106-109.
11. **Scott McVey, D., Kennedy, M., Chengappa, M.M.,** Veterinary Microbiology Set in 9/11pt ITC Stone Serif by AptaraR Inc., New Delhi, India, 2013.
12. **Stamatin, N.,** Microbiologie veterinară, Vol. II, Ed. Agro-Silvică de Stat, Bucureşti, 1957.
13. **Tasbac, B.A.,** Microbiologie veterinară generală. Lucrări practice ilustrate, Ed. a II-a, Editura Larisa, Campulung Muscel, 2018.
14. **Tasbac, B.A.,** Bacteriologie veterinară specială. Teorie şi Lucrări practice ilustrate, Editura Larisa, Campulung Muscel, 2016.
15. **Tasbac, B.A.,** A color handbook of special bacteriology. The Gram positive bacteria, Editura Larisa, Campulung Muscel, 2022.
16. **Togoe, I., Dobrea, M.,** Bacteriologie veterinară, Editura Printech, Bucuresti, 2006.
17. **Topley**, **W.W.C., Wilson, G.S.,** Topley and Wilson's Microbiology and Microbial Infections 10th Edition, Wiley, 2006.
18. **Public Health England**, UK Standards for Microbiology Investigations - Identification of Bacillus species.
19. ***https://www.tgw1916.net/Bacillus/cereus.html
20. ***https://www.tgw1916.net/Bacillus/mycoides.html
21. ***https://openresearchrepository.anu.edu.au/bitstream/1885/219768/4/Bacillus%20cereus%20Epidemiology%20Virulence%20Factors%20and%20Host%20Pathogen_AAM.pdf
22. ***https://www.njmonline.nl/getpdf.php?id=2132
23. ***https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/bacillus-anthracis
24. ***https://ro.wikipedia.org/wiki/Antrax
25. ***https://www.njmonline.nl/getpdf.php?id=2132

CYTOPATHOLOGY AND HISTOPATHOLOGY IN DIAGNOSIS OF MALIGNANT CUTANEOUS AND SUBCUTANEOUS MESENCHYMAL NEOPLASMS IN DOGS – A REVIEW

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Summary

Cutaneous and subcutaneous tumors have a high prevalence of 51% of all tumors in dogs, malignant mesenchymal (soft tissue) skin neoplasm representing about 20% to 40% of malignant tumors of the skin, as previously reported in recent studies. The aim of this present study is to summarise recent reported cytological and histopathological features of mesenchymal malignant cutaneous/subcutaneous tumors of dogs in order to give further aid for pathological differential diagnosis of these neoplasms. In case of canine skin pathology, mesenchymal neoplasms include malignant tumors of fibrous, adipose, vascular and perivascular tissues, smooth and striated muscle, nerve sheath, mesothelial and extra-skeletal chondro-osseous tumours. In addition, mast cell tumor, canine cutaneous histiocytoma and epitheliotropic lymphoma are included in malignant soft tissue tumors category, even some authors describe them as hematopoietic/lymphopoietic neoplasms. More prior studies have examined pathological findings of malignant soft tissue neoplasms affecting cutaneous and subcutaneous regions, mast cell tumor, histiocytoma, hemagiosarcoma being the most frequent malignant skin neoplasms noticed in canine skin pathology. Each malignant mesenchymal tumor in this study is classified in correlation with the third edition of International Histologic Classification of Tumors of Soft Tissues of Domestic Animals. After individually describing gross and microscopical aspects of the reviewed tumors, comparative cytopathological and histopathological findings are included in summary tables. Cytological and histological aspects may be useful to differentiate types of malignant soft tissue tumors, however, immunohistochemistry is required for identifying tumors subtypes.

Keywords: mesenchymal, tumors, skin, cytology, histopathology.

Mesenchymal tumors are described as neoplasms of soft tissue, which can evolve benign or malignant. In dogs, soft tissue tumors have been reported to be the third most common tumors. Most malignant soft tissue neoplasms are found in cutaneous and subcutaneous regions, being frequently noticed in canine pathology. As previously reported, mesenchymal tumors are the third most frequent tumors diagnosed in dogs, the prevalence of canine malignant soft tissue tumors being represented by about 20% to 40% of all malignant skin tumors outlined in canine pathology (7, 25).

As classified in Surgical Pathology of Tumors of Domestic Animals (3rd edition) – "World Health Organization" Tumor Classification (48), soft tissue (mesenchymal) malignant skin tumours described in dogs are represented by

tumors of fibrous tissue, adipose tissue, vascular and perivascular tissues, smooth and skeletal muscle, nerve sheat, mesothelial tumors and, extraskeletal chondro-osseous tumors. In addition, mast cell tumor, canine cutaneous histiocytoma and epitheliotropic lymphoma are included in malignant soft tissue tumors category, even some authors describe them separately as hematopoietic or lymphopoietic neoplasms. These tumors usually have minor cytological differences and sometimes even with histopathological examination it can be difficult to distinguish the mesenchymal cells origin.

In this article, gross morphology, cytopathological and histological aspects of each malignant mesenchymal tumors category will be outlined. For a complete diagnosis and an accurate prognostic in cases of neoplastic dermatopathologies, the pathologist needs as many clinical data as the veterinary clinician can provide about the case. Information that the veterinary pathologist needs might be reffering to breed, age, sex, previous treatments, blood chemistry panels, tumor localization or radiological findings.

Materials and methods

For the present review, plenty relevant articles published between 2005 and 2022 have been consulted. Soft tissue tumors classification was made in correlation with the third edition of International Histologic Classification of Tumors of Soft Tissues of Domestic Animals (48, 53), comparative cytological and histological aspects for each described tumor being outlined in content tables.

Results and discussions

After classifying malignant mesenchymal cutaneous and subcutaneous tumors of dogs, the most representative and commonly encountered neoplasms were described. For each reviewed tumor category, there were made individually gross, cytopathological (Giemsa stains) and histopathological (H&E stain) descriptions. Additional data regarding immunohistochemistry findings and malignancy grading were mentioned if necessary.

Malignant fibrous tumors

From this category, fibrosarcoma is considered to be one of the most frequent reported malignant soft-tissue tumors in dogs, representing up to 7.6% of all skin tumors in dogs (29). This sarcoma develops from fibroblasts and can occur cutaneous or subcutaneous. Previous studies show that fibrosarcoma has a wide age prevalence (from 2 years old dogs to geriatric specimens), but there is no breed-related occurrence reported (24, 65).

Regarding the gross usual findings, this tumour has, in most cases, a focal, but invasive distribution, being able to emerge as a firm mass anywhere on the

animal's body skin. Fibrosarcoma is hard to predict macroscopically because it can appear circumscribed or infiltrative, small or large (23, 33, 51).

In Table 1, there are summarized the most common aspects observed in cytological and histological examinations (Haematoxylin-Eosin stain) of the three types of skin fibrosarcomas: conventional (Fig. 1, Fig. 2), keloidal and myofibroblastic. Trichrome stains (e.g. Mallory) are often used for distinction of conjunctive tissue and for highlighting cellular pleomorphism (46).

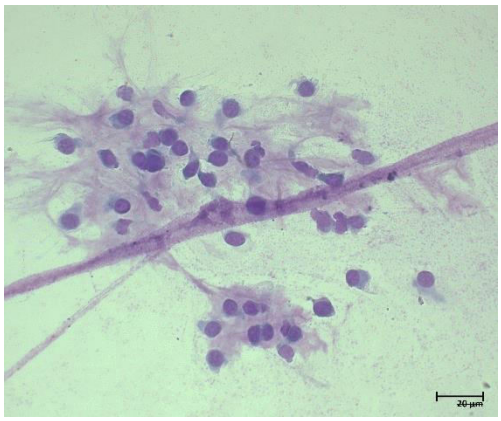

Fig. 1. Cytological aspects in fibrosarcoma, 200x, M.G.G. – spindle plumpy fibrocytes with basophilic cytoplasm (original)

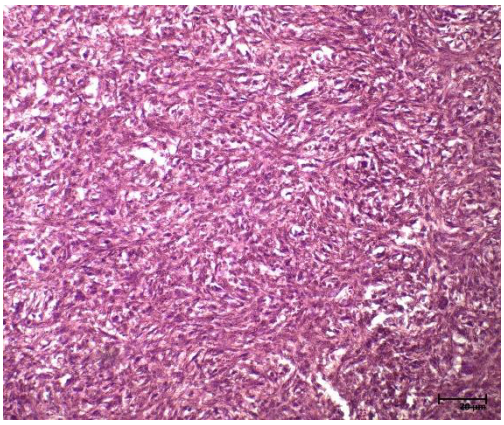

Fig. 2. Histological aspects in fibrosarcoma, 200x, H&E – fingerprint whorls aspect, spindle-shaped fibrocytes, collagen fibers (original)

Several previous studies about immunohistochemistry (IHC) findings showed that fibrosarcoma tumors express vimentin, Ki-67, COX2 and endosialin (CD248) (39, 44, 57, 65). In addition, fibrosarcomas are not expressing cytokeratin, SOX-10, Olig-2, GFAP (glial fibrillary acidic protein) or NGFR (nerve growth factor receptor) which can be helpful in differential diagnosis (48, 58).

Myxosarcoma

Also known as myxoid fibrosarcoma, this skin tumour has a fibroblastic origin, being characterised by the presence of mucin - a myxoid matrix rich in mucopolysaccharids (e.g. glycosaminoglycans) (23, 28, 59).

Tumoral lesions are frequently localised on the trunk and extremities cutaneous or subcutaneous tissue, being usually described as soft, poorly defined, pale/yellow lumps of various sizes. While sectioning, the masses might exude a gelatinous clear fluid (59).

In case of myxosarcoma, histological findings are the following: spindle-shaped cells (fibroblastic), fibrillar matrix, pleomorphic nucleus, moderate mitotic

activity. For differential diagnosis, Alcian-blue stain can be used for highlighting mucin intracytoplasmic vacuoles. In contrast, cytological aspects are not specific, but the pink linear arrangements of mucinous matrix can be suggestive (48).

Table 1

Cytological and histopathological aspects of fibrosarcomas

Type of fibrosarcoma	**Cytological findings (Giemsa stains)**	**Histopathological aspects (Haematoxylin-Eosin stain)**
Conventional fibrosarcoma (56, 57, 65)	• Usually more cellular than in fibroma • Collagen fragments • Large spindle atypical plumpy fibrocytes • Cellular binucleation, pleomorphism • Prominent multiple nucleoli • Basophilic cytoplasm Inflammatory cells are usually present	• Intersecting bundles • Fingerprint whorls aspect • Spindle shaped fibrocytes • Oval/elongated nuclei • Single/more prominent nucleoli • Abundant bright pink wavy collagen fibers • Low mitotic activity • Nuclear/cytoplasm ratio increased
Keloidal fibrosarcoma (41, 60)	• Pink/blue hyalinized collagen • Irregular spindle cells aggregate • Pink fibrillar matrix	• Fusiform cells in interlacing fascicles • Fibrovascular stroma • Hyalinized collagen • Few adipocytes • Central elongated nucleus • Low mitotic activity • Anisokaryosis, anisocytosis
Myofibroblastic fibrosarcoma (37, 48)	• Nonspecific	• Neoplastic cells less differentiated • Similar to conventional fibrosarcoma • Does express markers of smooth muscle differentiation (e.g. desmin, alpha-smooth muscle actin)

Malignant adipocytic tumors

The malignant adipose tissue tumor, known as liposarcoma, can appear in the subcutaneous region, being considered a rare tumor in middle-aged or older dogs (1% of all skin tumors in dogs) (7, 29).

Liposarcomas emerge from lipocytes and lipoblasts and can be classified in well-differentiated liposarcoma (also known as atypical lipomatous tumour), dedifferentiated liposarcoma, myxoid liposarcoma and pleomorphic type (5, 10, 47, 59). It was previously reported that the pleomorphic liposarcoma is highly metastatic (29). Mixed types have been previously reported (e.g. pleomorphic myxoid liposarcoma) (36). Microscopical aspects for differentiating this adipocytic tumor types have been outlined in Table 2. In case of myxoid liposarcoma, anastomosing capillaries, lipoblast and lipid intracellular vacuoles helps in differentiating this tumor from myxoid fibrosarcoma (32, 48).

In gross pathology, liposarcomas are described as firm, adherent, poorly capsulated or circumscribed, masses with high infiltrative behaviour, after sectioning a yellowish viscous material being observed inside (27, 29, 30).

Regarding other similar mesenchymal tumors, Sudan black and Oil red O can be used to differentiate liposarcoma, these special colorations highlighting lipid in cytological slides (48).

Immunohistochemical, liposarcomas express a positive reaction for vimentin and S-100 protein antibodies (38). In 2016, Avallone et al. (5) showed that MDM2 expression can be used for histological grading in case of well-differentiated and dedifferentiated liposarcomas in dogs.

Malignant vascular tumors

Hemangiosarcomas represent malignancies of vascular endothelial cells that can occur in the skin or subcutis of dogs, beside other known localisations (e. g. visceral hemangiosarcomas). It has been previously reported that cutaneous hemangiosarcomas represent 17% to 35% of all canine hemangiosarcomas (29).

Macroscopically, these cutaneous/subcutaneous hemangiosarcomas are presented as usually dark red multicentric soft masses that can be ulcerated due to disruption of affected blood vessels, being poorly circumscribed. These tumors are known for rapid growing and increased metastatic capacity (43).

These soft tissue malignant tumors can be staged in depending on the tumor invasion in tissue layers, classifying three tumoral stages: stage I – superficial hemangiosarcoma restricted to dermis area, stage II – tumors developing into subcutaneous tissues, stage III – tumors infiltrating muscle and fascia (29, 34).

Table 2

Liposarcomas cytological and histopathological characteristics

Type of liposarcoma	Cytological findings (Giemsa stains)	Histopathological aspects (Haematoxylin-Eosin stain)
Well-differentiated liposarcoma (27, 38, 48, 60)	• Moderate/high cellularity • Small clear lipid globules • Neoplastic round or spindle shaped vacuolated cells • Indistinct cell borders • Cells in large aggregates • Basophilic cytoplasm with clear vacuoles and large nuclei • Singular/multiple prominent nuclei • Moderate anisokaryosis and anisocytosis • Atypical mitosis • Anisocytosis • Atypical mitosis • High nuclear/cytoplasm ratio	• Multilobulated aspect • Fibrovascular stroma • Ovoid/polygonal adipocytes with cytoplasmic empty large lipid vacuoles • Atypical lipoblasts with hyperchromatic nucleus can be present, resembling lipoma • Oval/round dark nuclei with one visible compact nucleolus • Lipid vacuoles usually displace nuclei to periphery • Low mitotic activity • Necrosis areas and inflammatory infiltrate may be observed
Dedifferentiated liposarcoma (5)	• Nonspecific cytological aspects	• Mosaic pattern • Lipogenic islands • Non-lipogenic sarcoma areas • High mitotic activity in non-lipogenic areas
Myxoid liposarcoma (5, 60)	• Vacuolated mesenchymal cells • Areas of eosinophilic material • Mucinous extracellular matrix • Lipid vacuoles	• Round to stellate cells • Mucinous background • Rare lipoblasts • Rare neoplastic multinucleated cells • High mitotic activity • Occasional atypical mitosis • Anastomosing capillary bed
Pleomorphic liposarcoma (38)	• Nonspecific cytological aspects	• Atypical polygonal spindle cells • Eosinophilic optically clear cytoplasm • Multinucleation • Atypical mitosis • Rare lipoblasts • Atypical neoplastic adipocytes • Reduced lipid content • Necrotic areas • Fibrous stroma

Histologically, these tumors of the dog can be classified in 3 subtypes: conventional (Table 3), spindle-cell and epithelioid hemangiosarcomas (as in WHO soft tissue tumor classification) (48). In 2018, Bolfa et al. (8) described a rare cutaneous epithelioid hemangiosarcoma with granular cell differentiation (intracytoplasmatic granules duet to enzymatic deficiency in lysosomes). In addition, canine hemangiosarcomas can have different histological appearances, some authors classifying these tumors in morphologic subtypes: capillary, cavernous or solid subtypes (6, 32).

Cytological examination (Fig. 3) can be difficult in case of a hemangiosarcoma because of blood contamination on the slides, therefore histological aspects are more relevant for predicting a diagnosis (Fig. 4) (60).

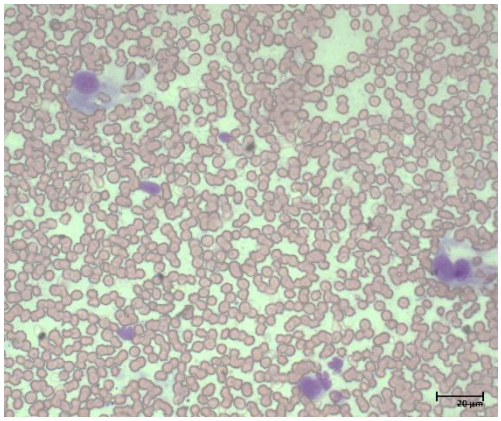

Fig. 3. Cytological aspects in hemangiosarcoma, 400x, M.G.G – fusiform/stellate mesenchymal cells, binucleation, intracytoplasmic clear vacuoles, blood contamination (original)

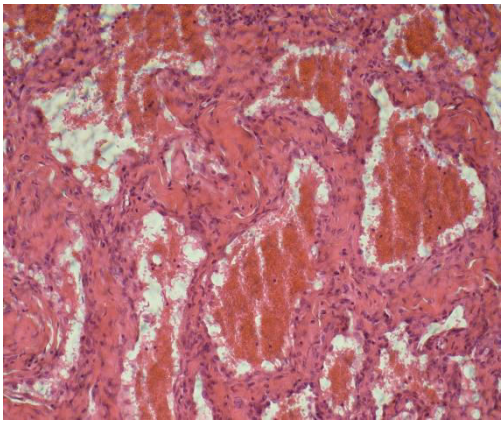

Fig. 4. Histological aspects in hemangiosarcoma, 200x, H&E – pleomorphic plumpy endothelial cells, irregularly anastomosing capillaries with erythrocytes and thrombi, collagen bundles (original)

It is important to differentiate hemangiosarcoma from other tumors with similar origin (e.g. hemangiopericytoma where a fingerprint pattern of fusiform cells can be observed) (57). For confirmation, in case of hemangiosarcomas, immunohistochemistry is required, this tumor being positive to CD31 marker (57). In addition, endothelial neoplastic cells express a positive reaction for vimentin vascular endothelial growing factor (VEGF) and Ki67 (31, 52).

Table 3

Conventional/ well-differentiated hemangiosarcoma cytological and histopathological aspects

Type of hemangiosarcoma	Cytological findings (Giemsa stains)	Histopathological aspects (Hemtoxylin-Eosin stain)
Conventional hemangiosarcoma (43, 52, 60)	• Blood contamination • Large neoplastic pleomorphic cells • Bi-/multinucleated cells • Spindyloid/ fusiform or stellate shaped cells • Nuclear:cytoplasm ratio law • Neoplastic cells have basophilic cytoplasm with few clear vacuoles • Erythrophagocytosis • Neutrophils and eosinophils may be present • Anisokaryosis and anisocytosis • Atypical mitosis	• Multilobulated aspect • Multiple irregularly anastomosing capillaries filled with erythrocytes and thrombi • Atypical/pleomorphic plumpy endothelial cells • Hyperchromatic large vesicular nuclei • Proeminent nucleoli • Cells aligned on collagen bundle • Erythrophagocytosis • Few mitotic aberant figures • Necrosis areas and inflammatory cells may be observed

Malignant peripheral nerve sheath tumors (PNSTs)

In this subcategory of mesenchymal cells tumors, the most representative neoplasia that can develop cutaneous in dogs is considered to be schwannoma (previously known as neurofibrosarcoma), even if some authors mention that the classification of peripheral nerve sheath tumors in veterinary medicine is not clarified yet (15, 48).

Histologically, these tumors present areas with high cellularity, disposed in palisade patterns where fusiform dark nuclei can be observed, resembling Antoni A schwannoma pattern described in human pathology (48). On the other hand, some cutaneous schwannomas in dogs are disposed in whorls patterns and have low cellular areas represented by thick, wispy cells, being similar to Antoni B pattern. Mixed patterns are observed often (48).

Cytological smears from malignant PNSTs are representated of proteinaceous and haemorrhagic background, spindle/oval cells with occasional intracytoplasmic vacuoles and malignant features (anisokaryosis, anisocytosis, multinucleation (15).

PNSTs might be differentiated from fibrosarcomas, myxosarcomas, benign nerve sheath tumors, therefor IHC evaluation can be perfomed, the following antibodies being used: PGP (protein gene product), GFAP, NGFR, laminin, collagen IV (48).

Canine mast cell tumor

In the past decades, mast cell tumor (or mastocytoma) was considered to be the most frequently diagnosed mesenchymal skin tumor in dogs (53), sometimes developing even in the subcutis (4, 9).These tumors are also known for highly metastatic predisposition and frequent recurrence (4). Even if the prevalence of mastocytoma is higher in older dogs (8 to 10 years old), it can be diagnosed also in young animals (9, 11, 55).

For histologically grading canine cutaneous mast cell tumors, there can be used two well-known grading systems: Patnaik three-tier system or Kiupel two-tier scheme (4, 9). In recent studies it is explained that these grading schemes should not be used for the diagnosis of subcutaneous tumors (4, 13, 26). Regarding cytological examination of mast cell tumors in dogs, it was described the Camus grade system which is the only known scheme that verifies granularity and cells binucleation. Camus grading system classifies tumors in high or low grade (4, 49). Other cytological grading systems were also described in literature (4, 26, 49).

General cytopathological and histological (Fig. 5, Fig. 6) aspects for mast cells tumors are described in Table 4. It must be mentioned that rapid Romanowski stains (e.g. Diff-Quick) do not always stain mast cell granules, therefore for a correct cytological examination Giemsa stains are recommended (Fig. 5) (14, 17).

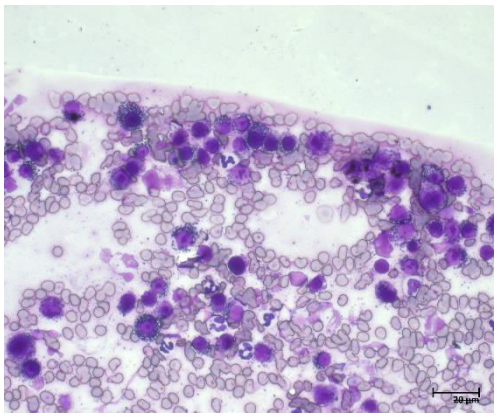

Fig. 5. Cytopathological aspects of mast cell tumor, 400x, M.G.G. – mast cells with metachromatic granules, degranulation, blood contamination (original)

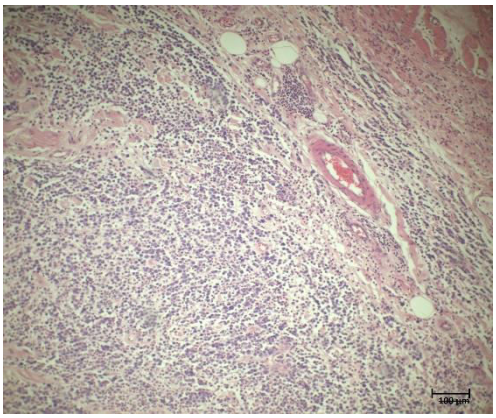

Fig. 6. Histopathological aspects of canine mast cell tumor, 100x, H&E – non-capsulated, infiltrative mass with pleomorphic round cells with mast cell morphology, anisokaryosis, anisocytosis and binucleation (original)

Subcutaneous mastocytomas in dogs have a lower prevalence, but they can be histologically classified into three patterns: circumscribed, infiltrative, mixed (17, 26, 63).

For IHC examination, in case of mast cell tumors, the most used markers are Ki-67 and KIT (or C117 which can express in three different patterns correlated with tumor prognosis) (2, 17).

Table 4

Mast cell tumor cytological and histopathological general aspects

Type of tumor	Cytological findings (Giemsa stains)	Histopathological aspects (Haematoxylin-Eosin stain)
Mast cell tumor (17, 19, 25, 57)	• Poorly/highly metachromatic granulated mast cells • Round and centrally placed nucleus • Anisokaryosis • Nuclear/cytoplasmic ratio high • Bi-/multinucleation can be observed • Mitotic figures can be present • Nuclear pleomorphism • Granules in the background • Eosinophils usually seen	• Highly cellularity • Cellular pleomorphism • Neoplastic cells with distinct borders • Numerous fine basophilic cytoplasmic granules • High mitotic index • Moderate to marked anisokaryosis • Histologic grading is necessary

Localized histiocytic sarcoma

Canine histiocytic sarcomas include localized and disseminated tumors, localized histiocytic sarcomas being described as round cell subcutaneous/cutaneous tumors originating from dermal dendritic cells (20, 22).

Table 5

Cutaneous histiocytic sarcoma cytological and histopathological aspects

Type of histiocytic proliferation	Cytological findings (Giemsa stains)	Histopathological aspects (Haematoxylin-Eosin stain)
Histiocytic sarcoma (20, 22, 42)	• Pleomorphic round/spindle cells • Round/oval nuclei • One/more nucleoli • Condensed chromatic • Basophilic cytoplasm and vacuolation • Bi-/multinucleation • Inflammatory infiltrate	• Large/vacuolated round cells • Plump mesenchymal cells • Multinucleated giant cells • Anisokaryosis, anisocytosis • High mitotic rate

Using IHC, this tumor must be negative for CD117, CD79 and CD3 stains, histiocytic sarcoma being compared with histiocytoma (which is a benign tumor) (12), mast cell tumor or cutaneous lymphoma (2). Dendritic cells origin of histiocytic sarcoma can be express through CD18 and MHC II (II major histocompatibility complex) immunohistochemical stains (22).

Cutaneous lymphoma

Lymphoma is described as a common malignant tumor in dogs, representing approximate 7-24% of all canine tumors as previously reported in recent studies (50). This neoplasm can affect dogs regardless of age or breed (61).

Based on WHO tumor classification, canine lymphomas can be easily classified in two major types: B-cell or T-cell lymphomas, the most common subtype diagnosed in dogs being diffuse large B-cell lymphoma (3, 61). In case of skin localisation, lymphomas can be divided in epitheliotropic (also known as 'mycosis fungoides') and non-epitheliotropic types, B-cell epitheliotropic lymphoma being diagnosed extremely rare in dogs (16, 23).

Grossly, epitheliotropic lymphomas are frequently described as erythematous, alopecic, ulcerated cutaneous lesions. In contrast, non-epitheliotropic lymphoma evolves commonly with cutaneous or subcutaneous nodules. Lymphadenopathy can be noted (23).

For a presumptive diagnosis, fine needle aspiration (FNA) from affected areas or lymph nodes can be a useful tool when suspecting canine lymphoma. Cytologic examination is helpful for identifying a round-cell malignant proliferation involving lymphoid cells with mitotic aberrant figures, lymphoglandular bodies and multinucleation (16, 60, 66).

Skin biopsy and even neoplastic lymph nodes excision histological examinations are required in order to distinguish the type of lymphoma and for tumoral grading. In case of epitheliotropic lymphomas, neoplastic lymphocytes frequently infiltrate all epithelial layers and malignant aspects are present: anisocytosis, anisokaryosis, mild mitotic index with atypical figures (23, 35, 60). 'Pautrier's' microaggregates are often encountered (23). Non-epitheliotropic lymphomas are mainly affecting superficial derm or subcutaneous area, resembling circumscribed non-capsulated nodules with neoplastic round-cells proliferation, where adnexal structures are rarely involved (16, 23, 54). Anisocytosis, anysokaryosis, multinucleation, mild mitotic activity and nuclear polymorphism are representative for this type of subcutaneous lymphoma (23).

Immunohistochemistry diagnosis of cutaneous T-cell lymphomas and, as well as differentiating epitheliotropic type from non-epitheliotropic lymphoma, can be made with CD3, CD18 and CD79a staining (2). B-cells lymphoma express CD79a, CD18 and MHC II (2, 16).

Transmissible venereal tumor (TVT/Sticker sarcoma)

TVT represents a malignant mesenchymal tumor specific to individuals from *Canidae* family which is transmitted by direct transfer of tumoral cells between hosts (21, 62). In Romania, there are reported numerous cases of this tumor, due to lack of neutering in stray dog populations (21, 62).

This tumor can affect both sexes, the most frequent localisation being on the genitalia, but extragenital localised (mucosal or cutaneous) TVT is frequently encountered (1, 40).

In gross pathology, Sticker sarcomas resemble proliferative cauliflower-like red friable masses, nodular or multilobulated, ulcerated in most cases (1,40).

Cytopathological findings (Fig. 7) show pleomorphic round cells (lymphocytoid type cells), round to ovoid nuclei located centrally with anisokaryosis, slightly vacuolated cytoplasm and presence of frequent atypical mitotic figures (1, 21, 60).

Histologically (Fig. 8), TVTs present round cells arranged in rows or cords with large pleomorphic nuclei, centrally placed nucleoli and vacuolated cytoplasm. The fibrous stroma is well represented, new blood vessels are usually seen and the mitotic index is high (1, 21, 40, 64).

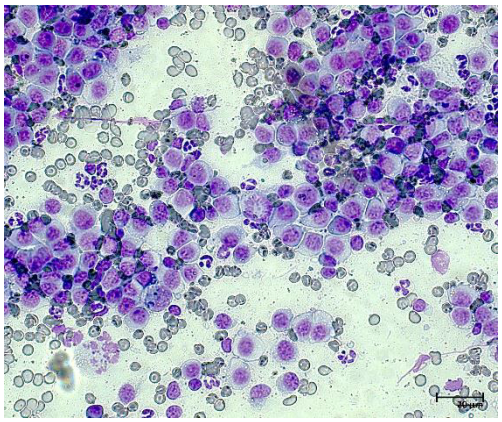

Fig. 7. Cytological aspects in TVT, 200x, M.G.G. - pleomorphic round cells, slighty vacuolated cytoplasm, atypical mitotic figures (original)

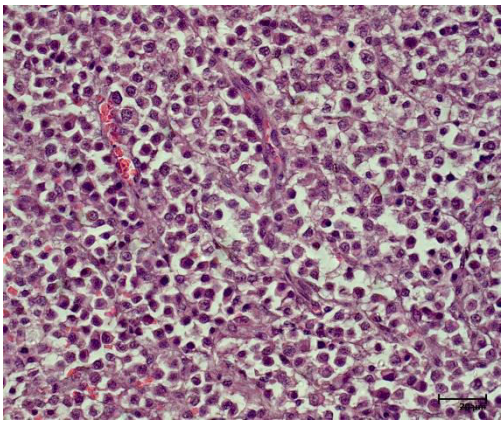

Fig. 8. Histological aspects in TVT, 400x, H&E – lymphocytoid (round) cells arranged in cords, large nuclei, fibrous stroma, new blood vessels (original)

For a differential diagnosis, this tumor can be differentiated from lymphoma, histiocytoma, undifferentiated carcinoma or amelanotic melanoma using immunostainings (40, 45). As previously reported by Park et co., 2006 (45), transmissible venereal tumors have a positive reaction with vimentin and Ki-67, but negative staining for CD3 and CD79 (21, 40).

Conclusions

FNA cytologic examination represents an affordable and practical technique for presumptive diagnosis in case of canine malignant cutaneous and subcutaneous malignant tumors. This examination has been previously recognized as a useful tool for identifying cellular origin, differentiating benign from malignant proliferations, and, in some cases (e.g. mast cell tumor), even for grading the tumor.

However, in many instances, cytology is not revealing enough information for an effective diagnosis, histological aspects being important for classifying the involved neoplasm, displaying tumor invasion capacity and implementing the therapeutic scheme.

Furthermore, in certain cases, immunohistochemistry is currently used for differentiating between similar tumors, subtyping malignant neoplasms and for grading systems.

In summary, cytological and histological examination have evolved in the last decade, correlation of these diagnosis techniques with clinical examination, gross morphology, different laboratory tests and, moreover, immunohistochemistry is efficient for obtaining a confirmatory diagnosis of the canine cutaneous or subcutaneous mesenchymal malignant tumors.

References

1. **Abeka, Y.T.,** Review on Canine Transmissible Venereal Tumor (CTVT), Cancer Therapy and Oncology International Journal, 2018, 14, 4, 555895.
2. **Araújo, M.R., Preis, I.S., Lavalle, G.E., Cassali, G.D., Ecco, R.,** Histomorphological and immunohistochemical characterization of 172 cutaneous round cell tumours in dogs, Pesquisa Veterinária Brasileira, 2012, 32, 8, 772-780.
3. **Aresu, L.,** Canine Lymphoma, More Than a Morphological Diagnosis: What We Have Learned About Diffuse Large B-cell Lymphoma, Frontiers in Veterinary Sciences, 2016, 3, 77.
4. **Avallone, G., Rasotto, R., Chambers, J.K., Miller, A.D., Behling-Kelly, E., Monti, P., Berlato, D., Valenti, P., Roccabianca, P.,** Review of Histological Grading Systems in Veterinary Medicine, Veterinary Pathology, 2021, 1-20.
5. **Avallone, G., Roccabianca, P., Crippa, L, Lepri, E., Brunetti, B., Bernardini, C., Forni, M., Olandese, A., Sarli, G.,** Histological Classification and Immunohistochemical Evaluation of MDM2 and CDK4 Expression in Canine Liposarcoma, Veterinary Pathology, 2016, 53, 4, 773-780.
6. **Avallone, G., Stefanello, D., Ferrari, R., Roccabianca, P.,** The controversial histologic classification of canine subcutaneous whorling tumours: The path to perivascular wall tumours, Veterinary Comparative Oncology, 2020, 18, 1, 3-8.

7. **Baba, Al., Catoi, C.,** Comparative Oncology, The Publishing House of the Romanian Academy, Bucharest, 2007.
8. **Bolfa, P., DellaGrotte, L., Weronko, T., Armien, A.G.,** Cutaneous epithelioid hemangiosarcoma with granular cell differentiation in a dog: a case report and review of literature, Journal of Veterinary Diagnostic Investigation, 2018, 1, 4, 951-954.
9. **Cavalcante, I.M.S., Bernardes, F.C.L, Freire, B.S, Cuconati, R., Munhoz, J., Raposo, C.,** A retrospective study of canine cutaneous mast cell tumor: correlation between clinical, histological and molecular characteristics, Brazilian Journal of Development, 2020, 6, 12, 100281-100299.
10. **Cibas, E.S., Ducatman, B.S.,** Cytology – Diagnostic Principles and Clinical Correlated, Third Edition, Elsevier, Missouri, 2009.
11. **Cifuentes-Arias, S., Osorio-Morales, L., Pedraza-Ordonez, F.,** Clinical follow-up of canine mast cell tumour cases diagnosed by cytology and histopathology, Veterinarska Stanica, 2021, 52, 4, 397-403.
12. **Ciobotaru, E., Militaru, M., Soare, T., Mocanu, J., Bârţoiu, A.,** Canine cutaneous histiocytoma: morphology and morphometry, Veterinary Dermatology, 2004, 15, 1, 62-68.
13. **Curtseit, S., Condruţ, E., Leonardi, L., Militaru, M.,** A poorly differentiated metastatic mast cell tumor and secondary lesions in dog. Case presentation, Revista Română de Medicină Veterinară, 2013, 23, 3, 79-90.
14. **Curtseit, S., Militaru, M.,** Canine cutaneous mast cell tumor. Etiopathogenesis, diagnosis, clinical staging and prognosis, Revista Română de Medicină Veterinară, 2013, 23, 3, 27-42.
15. **Da Costa, R.C., Parent, J.M., Dobson, H., Ruotsalo, K., Holmberg, D., Duque, C., Polma, R.,** Ultrasound-guided fine needle aspiration in the diagnosis of peripheral nerve sheat tumors in 4 dogs, Canadian Veterinary Journal, 2008, 49, 1, 77-81.
16. **De Bosschere, H., Declercq, J.,** Cutaneous nonepitheliotropic B-cell lymphoma in a Golden retriever, Vlaams Diergeneeskundig Tijdschrift, 2008, 77, 315-318.
17. **de Nardi, A.B., dos Santos Horta, R., Fonseca-Alves, C.E., de Paiva, F.N., Linhares, L.C.M., Firmo, B.F., Ruiz Sueiro, F.A., de Oliveira, K.D., Lourenço, S.V., De Francisco Strefezzi, R., Brunner, C.H.M., Rangel, M.M.M., Jark, P.C., Castro, J.L.C., Ubukata, R., Batschinski, K., Sobral, R.A., da Cruz, N.O., Nishiya, A.T., Fernandes, S.C., dos Santos Cunha, S.C., Gerardi, D.G., Challoub, G.S.G., Biondi, L.R., Laufer-Amorim, R., de Oliveira Paes, P.R., Lavalle, G.E., Huppes, R.R., Grandi, F., de Carvalho Vasconcellos, C.H., dos Anjos, D.S., Luzo, Â.C.M., Matera, J.M., Vozdova, M., Dagli, M.L.Z.,** Diagnosis, Prognosis and Treatment of Canine Cutaneous and Subcutaneous Mast Cell Tumors, Cells, 2022, 11, 618.
18. **Dinescu, G., Ciobotaru, E., Curtseit, S., Gubceag, E., Feghiu, A., Militaru, M.,** Overview of epidemiology and morphological diagnosis of cutaneous

lesions in dog, Lucrări Științifice - Universitatea de Științe Agricole a Banatului Timișoara, Medicină Veterinară, 2011, 44, 2, 56-61.
19. **Dinescu, G., Curtseit, S., Ciobotaru, E., Soare, T., Militaru, D., Militaru, M.,** Elements of the Epidemiology of Canine Mast Cell Tumour diagnosed by Cytological Examination, Journal of Comparative Pathology, 2010, 4, 143, 318.
20. **Dinescu, G., Fundățianu, I.C., Tănase, A., Condruț, E., Soare, T., Militaru, M.,** Cytological Diagnosis of Canine Cutaneous Histiocytic Proliferative Disorders, Scientific Works. Series C, Veterinary Medicine, 2016, LXI, 2, 123-126.
21. **Ferghiu, A., Crînganu, D., Dumitrescu, F., Pascal, M., Vasilescu, F., Militaru, M.,** Nasal transmissible venereal tumor in a jack russel terrier bitch, Acta Veterinaria-Beograd, 2015, 65, 1, 143-148.
22. **Fulmer, A.K., Mauldin, G.E.,** Canine histiocytic neoplasia: An overview, Canadian Veterinary Journal, 2007, 48, 1041-1050.
23. **Gross, T.L., Ihrke, P.J., Walder, E.J., Affolter, V.K.,** Skin Diseases of the Dog and Cat, Second Edition, Wiley-Blackwell, 2005.
24. **Hassan, B.B., Al-Mokaddem, A.K., Abdelrahman, H.A., Samir, A., Mousa, M.R.,** Cutaneous Tumors in Dogs: A Retrospective Epidemiologica land Histological Study of 112 Cases, Advances in Animal and Veterinary Sciences, 2022, 10, 1, 170-182.
25. **Hohenhaus, A.E., Kelsey, J.L, Haddad, J., Barber, L., Palmisano, M., Farrelly, J., Soucy, A.,** Canine Cutaneous and Subcutaneous Soft Tissue Sarcoma: An Evidence-Based Review of Case Management, Journal of the American Animal Hospital Association, 2016, 52, 2, 77-89.
26. **Hosseine, E., Pedram, B., Bahrami, A.M., Moghaddam, M.H.J, Javanbakht, J., Ghomi, F.E., Moghaddam, N.J., Koohestani, M., Shafiee, R.,** Cutaneous mast cell tumor (Mastocytoma): Cyto- histopathological and haematological investigations, Diagnostic Pathology, 2014, 9.
27. **Indika, K.G., Kumara, M.P. Ariyarathna, H.M.H.S, Jayasinghe, M.G.C.M., Adikari, A.M.R.B., de Siva, D.D.N.,** Two cases of canine liposarcomas: an uncommon tumor, Sri Lanka Veterinary Journal, 2016, 63, 1, 23-26.
28. **Iwaki, Y., Lindley, S., Smith, A., Curran, K.M., Looper, J.,** Canine myxosarcomas, a retrospective analysis of 32 dogs (2003-2018), BMC Veterinary Research, 2019, 15, 217.
29. **Johnston, S.A., Tobias, K.M.,** Veterinary Surgery Small Animal, Second Edition, Elsevier, Missouri, 2018.
30. **Julie, B., Abraham, S.S., Jayesh, V., Raghavan, K.S.,** Pathological observations on liposarcoma in a dog, Indian Journal of Veterinary Pathology, 2012, 36, 1, 97-98.
31. **Karabagli, G., Duzgun, O., Yildar, E., Erdogan, O., Gurel, A.,** A Hemangiosarcoma Case in a Dog, Journal of Faculty of Veterinary Medicine, Istanbul University, 2011, 37, 2, 161-165.

32. **Karnik, M.K., Anjankumar, K.R., Gowda, Y., Rakshith, K., Shettar, M., Azeemullah, H.R., Yashas, R.K., Rajashekaraiah, R., Mahesh, V., Rao, S., Satyanaryana, M.L.,** Incidence and Histopathological Studies on Tumours of Dog in Bengaluru, India, International Journal of Current Microbiology and Applied Sciences, 2020, 9, 2, 747-752.
33. **Kashyp, D.K., Tiwari, S.K., Giri, D.K., Dewangan, G., Sinha, B.,** Cutaneous and subcutaneous tissue neoplasm in canines: Occurrence and histopathological studies, African Journal of Agricultural Research, 2013, 8, 49, 6569-6574.
34. **Kim, J.H., Graef, A.J., Dickerson, E.B., Modiano, J.F.,** Pathobiology of Hemangiosarcoma in Dogs: Research Advances and Future Perspectives, Veterinary Sciences, 2015, 2, 4, 388-405.
35. **Lam, A.,** Cutaneous lymphoma in a Dachshund, Canadian Veterinary Journal, 2021, 62, 7, 771-774.
36. **Lee, E.J, Jeong, K.S.,** Histopathological Features of Myxoid Pleomorphic Liposarcoma in an African Pygmy Hedgehog (*Atelerix Albiventris)*, Veterinary Sciences, 2022, 9, 11, 642.
37. **Liu, S.M., Mikaelian, I.,** Cutaneous Smooth Muscle Tumors in the Dog and Cat, Veterinary Pathology, 2003, 40, 6, 685-692.
38. **Makovicky, P., Juhasz, A., Rimarova, K., Makovicky, P.,** Histological Aspects of White Fatty Lesions in Dogs: Case Reports, International Journal of Applied Research in Veterinary Medicine, 2019, 17, 2, 41-46.
39. **Marzec, M., Kandefer-Gola, M., Janus, I., Bubak, J., Nowak, M.,** Endosialin (CD248) Expression in Fibromas and Soft-tissue Fibrosarcomas in Dogs, In vivo, 2021, 35, 3, 1457-1472.
40. **Meuten, D.J.,** Tumors in Domestic Animals, Fifth Edition, Wiley-Blackwell, 2017.
41. **Mikaelian, I., Gross, T.L.,** Keloidal Fibromas and Fibrosarcomas in the Dog, Veterinary Pathology, 2016, 39, 1, 149-153.
42. **Moore, P.F.,** A review of Histiocytic Diseases of Dogs and Cats, Veterinary Pathology, 2014, 5, 1, 167-184.
43. **Mullin, C., Clifford, C.A.,** Histiocytic Sarcoma and Hemangiosarcoma Update, Veterinary Clinics of North America: Small Animal Practice, 2019, 48, 855-879.
44. **Nagendra Reddy, T., Amaravathi, P.,** Rapid Diagnosis of Fibrosarcoma in a Dog using Fine Needle Aspiration Cytology (FNAC), Biological Forum – An International Journal, 2021, 13, 3, 174-176.
45. **Park, M.S., Kim, Y., Kang, M.S., Oh, S.Y., Cho, D.Y., Shin, N.S., Kim, D.Y.,** Disseminated Transmissible Venereal Tumor in a Dog, Journal of Veterinary Diagnostic Investigation, 2006, 18, 1, 130-133.
46. **Ribeiro, F.T.L, Assef, M.L.M., Cirio, S.M.,** Histopathological study of Cutaneous Fibrosarcoma in Dogs (*Canis familiares,* Linnaeus, 1758) with

different staining techniques, Rev. Acad., Ciênc. Agrár. Ambient., Curitiba, Exactas, 2009, 7, 3, 341-347.
47. **Roccabianca, P.,** Canine Skin Cancer and the Art of Classification, Veterinary Pathology, 2018, 55, 6, 770-771.
48. **Roccabianca, P., Schulman, F.Y., Avallone, G., Foster, R.A., Scruggs, J.L., Dittmer, K, Kiupel, M.,** Surgical Pathology of Tumors of Domestic Animals, Volume 3: Tumors of Soft Tissue, Davis-Thompson Foundation, Illinois, 2020.
49. **Salvi, M., Molinari, F., Iussich, S., Muscatello L.V., Pazzini, L., Benali, S., Banco, B., Abramo, F., De Maria, R., Aresu, L.,** Histopathological Classification of Canine Cutaneous Round Cell Tumors Using Deep Learning: A Multi-Center Study, Frontiers in Veterinary Medicine, 2021, 14, 20, 5008.
50. **Sanchez, D., Sanchez-Verin, R., Corona, H., Gutierrez, A., Nunez-Ochoa, L., Paredes, J., Cesarman-Maus, G.,** Canine lymphoma: Pathological and clinical characteristics of patients treated at a referral hospital, Veterinaria Mexico OA, 2019, 6, 2, 43.
51. **Sanchez-Redondo, S., Hare, C.H.Z., Constantino-Casas, F., Williams, T.L.,** Correlation between cytological features and histologic grades in cutaneous and subcutaneous soft tissue sarcomas in dogs – A pilot study, Veterinary Clinical Pathology, 2021, 50, 2, 236-239.
52. **Santos dos Anjos, D., Assis, A.R., Fronseca-Alves, C.E., Babo-Terra, V.J.,** Clinical, Pathological and Immunohistochemical Evaluation of a Primary Hemangiosarcoma in a Pinscher Dog, Arta Scientiae Veterinariae, 2016, 44, 1, 125.
53. **Sharif, M.A.M.,** Epidemiology of skin tumor entities according to the new WHO classification in dogs and cats, VVB Laufersweiler Verlag, Giessen, 2006.
54. **Siepmann, E.C., Tanabe, L.Y., Cestari, F.K., Druziani, J.T., de Marco Viott, A.,** Nonepitheliotropic lymphoma in a dog: a case report, Medicina Veterinaria (UFRPE), 2020, 14, 1, 33-37.
55. **Simkus, D., Pockevicius, A., Maciulskis, P., Simkiene, V., Zorgevica-Pockevica, L.,** Pathomorphological Analysis of The Most Common Canine Skin and Mammary Tumours, Veterinarija ir Zootechnika, 2015, 69, 91, 63-70.
56. **Soujanya, S., Madhuri, D.,** Cutaneous fibrosarcoma in a dog, Journal of Entomology and Zoology Studies, 2019, 7, 1, 861-863.
57. **Subapriya, S., Pazhanivel, N., Gokulakrishnan, M, Nagarajan, B., Kavitha, S., Sumathi, D., Vairamuthu, S.,** Incidence and pathology of skin tumours in dogs, Pharma Innovation Journal, 2021, 10, 5, 620-629.
58. **Subapriya, S., Vairamuthy, S., Pazhanivel, N., George, R.S., Vijayarani, K., Gokulakrishnan, M.,** Histopathological and Immunohistochemical Diagnosis of Canine Fibrosarcoma, International Journal of Current Microbiology and Applied Sciences, 2018, 7, 6, 1376-1379.

59. **Vail, D.M., Thamm, D.H., Liptak, J.,** Withrow and MacEwen's Small Animal Clinical Oncology, 6th Edition, Elsevier, Missouri, 2020.
60. **Valenciano, A.C, Cowell, R.L.,** Cowell and Tyler's Diagnostic Cytology and Hematology of the Dog and Cat, Fifth Edition, Elsevier, Missouri, 2020.
61. **Valli, V.E., San Myint, M., Barthel, A., Bienzle, D., Caswell, J., Colbatzky, F., Durham, A., Ehrhart, E.J., Johnson, Y., Jones, C., Kiupel, M., Labelle, P., Lester, S., Miller, M., Moore, P., Moroff, S., Roccabianca, P., Ramos-Vara, J., Ross, A., Scase, T., Tvedten, H., Vernau, W.,** Classification of Canine Malignant Lymphomas According to the World Health Organization Criteria, Veterinary Pathology, 2011, 48, 1, 198-211.
62. **Vițalaru, B.A., Crânganu, D., Polter, G.,** Comparative therapeutic approach of canine transmissible venereal tumors (TVT), Scientific Works, Series C, Veterinary Medicine, 2012, 58, 398-405.
63. **William, M., Yuzbasiyan-Gurkan, V., Marconato, L., Dacasato, M., Hadzijusufovic, E., Hermine, O., Safovnik, I., Gamperl, S., Schneeweiss-Gleixner M., Gleixner K., Bohm T., Peter B., Eisenwort G., Moriggi, R., Li, Z., Jawhar, M., Sotlar, K., Jensen-Jarolim, E., Sexl, V., Horny, H.-P., Galli. S.J., Arock. M., Vail, D.M., Kiupel, M., Valent, P.,** Proposed Diagnostic Criteria and Classification of Canine Mast Cell Neoplasm: A Consensus Proposal, Frontier in Veterinary Science, 2021, 8, 755258.
64. **Yadav, A., Kumar, P., Panihar, S., Mehra, U.S., Bugalia, N.S., Yadav, R., Kumar, P.,** Clinico-Histopathological Studies in Canine Transmissible Venereal Tumour, International Journal of Current Microbiology and Applied Sciences, 2018, 7, 11, 2714-2719.
65. **Yoo, S., Kim, H.J., Kim, H.J., Lee, S.Y., Kim, S.H., Yoo, Y.S., Hwang, J.Y., Do, S.H., Kim, H.Y., Kim, D.H.,** Subcutaneous Fibrosarcoma in the Occipital Region with Nuchal Crest Adhesion in a 5-month-old Dog, Journal of Veterinary Clinics, 2018, 32, 2, 63-66.
66. **Zandvliet, M.,** Canine lymphoma: a review, Veterinary Quarterly, 2016, 36, 2, 76-104.

PREVALENCE OF *MICROSPORUM* SPP. IN FELINE POPULATIONS FROM A SHELTER IN WESTERN ROMANIA

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Summary

Dermatophytosis, a zoonotic disease caused by species of the *Microsporum* genus, continues to be an important subject in veterinary and human dermatology, due to its increased prevalence. In cats, *Microsporum* spp. causes a skin disease, characterized by circular, alopecic, crusty and erythematous lesions. However, it is well-known that many cats can act as asymptomatic carriers. The aim of this study was to evaluate the prevalence of *Microsporum* spp. among shelter cats, with or without suggestive clinical signs, to determine the extent to which this zoonotic disease is spread among the population of adoptable cats, which, in turn, will continue to spread it to their possible future owners, and to bring new insight into the epidemiology of the disease with the final scope of aiding the staff from shelters to properly apply prevention and treatment methods. The study was conducted on 40 cats from a shelter located in Băile Herculane, Caraș-Severin County, from January to May 2022. Hairs were collected from cats equally divided into two categories: cats with suggestive clinical signs (n=20) and asymptomatic cats (n=20). The hairs were transferred on dermatophyte specific culture media (DTM), followed by a microscopic exam of the resulting cultures in order to confirm the presence of *M.canis*. The results indicated a significant prevalence rate of this dermatophyte among the shelter cat population (48%), with no significant differences based on sex but with significant differences based on age (68% positivity in cats under the age of 1 year) as well as in cats displaying clinical signs (85% positivity) as compared to asymptomatic cats). This high prevalence rate leads to the conclusion that shelter cats represent a significant hazard in the spreading of this zoonotic disease, not only to other shelter cats, but also posing a risk for their future adoptive families and other household pets.

Keywords: *Microsporum* spp, shelter cats, asymptomatic, prevalence.

Dermatophytosis, commonly known as ring worm, is a highly disputed matter in the world of both veterinary and human dermatology, hence its zoonotic character and its high frequency in the western hemisphere (8, 11). The most common species of dermatophyte affecting felines is *Microsporum canis*, a pathogen that can cause important cutaneous clinical signs such as alopecia, crusting, erythema and pruritus (4). Many felines however, only act as asymptomatic carriers, with the disease becoming clinically apparent only in immune-suppresed animals (3). Several favoring factors such as crowding, poor hygiene, outdoor access, compromised immune status or stress, facilitate the evolution of this condition.

Thus, animal shelters become the ideal places for the proliferation of these pathogens among felines which in the end, get adopted and spread the disease

further on.

Materials and methods

Study subjects

The study was carried out on 40 cats from a shelter located in Băile Herculane Caraș-Severin County, between January and May 2022.

The animals taken into study were stray cats, aged between 2 Female and 9 years, of different sexes (22 females and 18 males), most of them being European breed (31), 3 Siamese cats, 2 British shorthair cats and 4 Burmese breed cats. The cases representing the source of material for the study were divided into two equal categories, i.e. 20 of the cats had no visible dermatological lesions and the other half of 20 cats had various dermatological lesions (alopecia, scales, scabs) located on various areas of the head, neck or limbs. Details on the description of each case are given in Table 1.

Table 1

Detailed description of the studied cases

No	Breed	Age	Sex	Lesions
1	Common breed	2 years	Female	None
2	Common breed	7 months	Male	None
3	Burmese	6 years	Male	None
4	British shorthair	4 years	Female	None
5	Common breed	3 months	Female	None
6	Common breed	3 months	Male	None
7	Siamese	2 years	Female	None
8	Common breed	1 year	Female	None
9	Common breed	1,5 years	Male	None
10	Common breed	1 year	Male	None
11	Burmese	9 months	Female	None
12	Common breed	5 months	Female	None
13	British shorthair	4 months	Female	None
14	Common breed	1 year	Male	None
15	Common breed	1 year	Male	None
16	Common breed	5 months	Male	None
17	Common breed	2 years	Female	None
18	Common breed	1 year	Female	None
19	Common breed	9 years	Male	None
20	Common breed	2 years	Male	None
21	Common breed	8 months	Male	Periocular and ear alopecia, scales
22	Common breed	6 months	Male	Head and ear alopecia

23	Common breed	1 year	Male	Crsuting and alopecia, head area
24	Common breed	1 year	Male	Alopecia in the head an limbs area
25	Common breed	2 years	Female	Alopecia in the head and limbs
26	Common breed	7 months	Female	Alopecia in the head and limbs,erythema
27	Common breed	1 year	Female	Scales and alopecia
28	Common breed	2 years	Male	Alopecia in the head and neck, erythema
29	Siamese	2 months	Female	Alopecia and crusting of the head and neck
30	Siamese	5 months	Female	Alopecia in the head area
31	Common breed	1,5 years	Female	Alopecia, erythema
32	Common breed	1 year	Female	Alopecia and erythema in the head and limbs
33	Common breed	3 years	Male	Scales in the ear area, head alopecia, erythema
34	Common breed	2 years	Male	Alopecia and erythema
35	Common breed	1 year	Male	Alopecia of the heaf and external neck
36	Common breed	7 months	Female	Periocular and ear alopecia, scales, erythema
37	Common breed	6 months	Female	Alopecia in the periocular area ,ears and scales

38	Common breed	9 months	Female	Scales in the ear area and alopecia, erythema
39	Common breed	3 years	Female	Alopecia and crusts in the head and limbs area
40	Common breed	2 years	Female	Periocular alopecia and scales

Sampling Methods and Cultures

For the study, hairs were sampled from cats with lesions, as well as from those without visible dermatological lesions, but kept in the same space as those visibly affected.

The hairs from each sample were seeded on Sabouraud medium with added antibiotic, poured into Petri dishes, after previously being kept for 1 minute in medical alcohol to reduce the bacterial load (Fig 2).

Fig. 1. Sampled hairs ready to be seeded on a culture medium (original)

The Sabouraud medium was prepared in the Mycotic Diseases Clinic of the Faculty of Veterinary Medicine, Timisoara. One litre Sabouraud consists of: 10 g peptone, 20 g agar, 40 g glucose.

Method of preparation: 10 g peptone is dissolved in 1 l distilled water, heated to 80°C, allowed to return to room temperature, to which 20 g agar is added, brought to boiling point (100°C), allowed to return to room temperature, to which 40 g glucose is added. Check the pH (pH BOX, Merck), which should be between 5.8 and 6.2, then dispense into flasks and autoclave at 115-120°C for 15 minutes (4).

The seeded plates (Fig. 2) were placed in an incubator at 25 °C and kept there for 7 days. From the second day after inoculation, the plates were examined

daily to observe colony development.

After the 7 days, colonies that had macroscopic characters similar to those produced by *Microsporum* were examined under a microscope, with aniline blue microscopic slides.

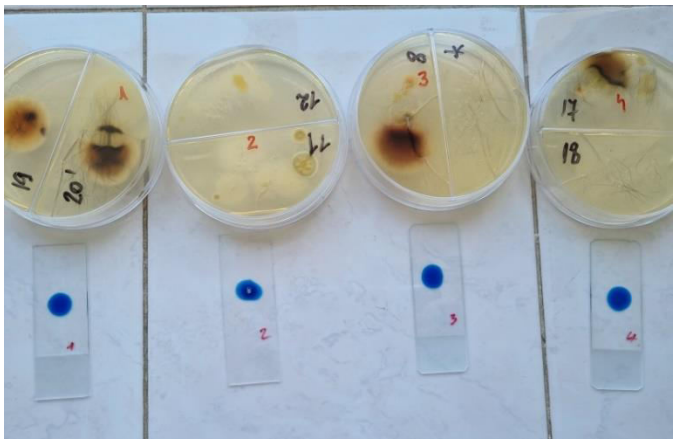

Fig. 2. Cultured media and microscope slides stained with aniline blue (original)

Colonies that on microscopic examination showed microscopic characteristics specific to the genus *Microsporum* (septate hyphae with microconidia and fusiform macroconidia with 5-15 cells with a rough surface, a thick outer wall and a terminal sheath) were transplanted onto DTM (Dermatophyte Test Medium), which also helps to confirm the presence of the *Microsporum* genus (Fig. 3).

Fig. 3. DTM plate with a *Microsporum spp.* species colony (original)

The plates were incubated at 25 °C for 5-7 days and the development of white, fluffy colonies that induce a yellow to red colour change in the medium confirms the presence of a dermatophyte pathogen of the genus *Microsporum*.

Results and discussions

The animals were divided into two categories in terms of lesions, i.e. animals without visible skin lesions and animals with visible skin lesions. In terms of age, two broad age groups were outlined as follows - under one year and over one year.

Since purebred individuals were present in far too small a number, it was not considered to study the distribution of positive cases according to breed.

Thus, out of 40 cats, 19 (48%) animals were positive for *Microsporum* spp.

Of the 40 examined cases, 22 were females and 18 males. The distribution of positive cases according to sex is as follows: 11 positive females (58% of the total number of positive cases) and 8 positive males (42% of the total number of positive cases) (Fig. 4).

In terms of the two age groups, the majority of positive cases were found in the under 1 year age group - 13 positive cases (68%) and 6 positive cases in the over 1 year age group (32%) (Fig. 5).

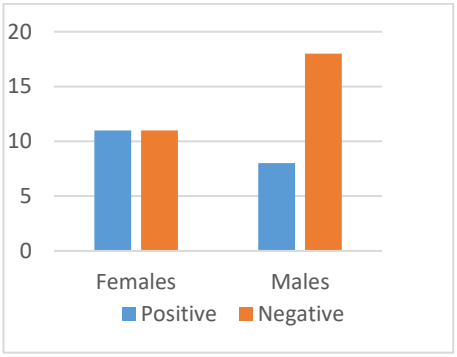

Fig. 4. Distribution of positive cases throughout the studied population

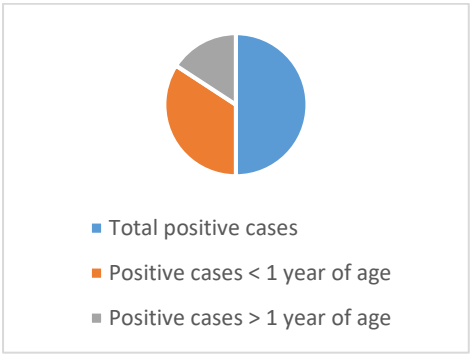

Fig. 5. Distribution of positive cases according to age

In terms of lesions, two of the cats (10%) in the lesion-free category (20 individuals) were culture positive and 17 (85%) of those with clinical dermatological signs (20 individuals) were culture positive.

Dermatophytoses are important zoonoses, accounting for up to 80% of fungal infections in rural humans and 20% of fungal infections in urban humans due to close contact between humans and their pets (20). It is very important to determine the prevalence rate of these mycoses in shelter populations in order to have a clearer picture of the risk of transmission of this zoonotic disease from animals adopted from

shelters to their owners.

The lack of concrete information on the prevalence of these fungi in animals, especially in animals without clinical signs, which are considered carriers, may hamper the treatment of these fungal infections (20).

A study in Brazil (2003)(1) conducted over a period of one year and on a similar number of cats revealed a prevalence rate of 36.8% of dermatophytes of the genus *Microsporum* spp.

Studies in the literature show a general prevalence of this fungus in feline populations between 9 and 46% depending on various factors, our results being in agreement with the maximum values identified by researchers - 48%, similar to studies carried out in other European countries such as Croatia (40.7%)(13), Italy (24.7%)(4), France (22%)(12).

The literature shows a higher prevalence of positive cases in the under one year age group, similar to our results (68% versus 32%). This distribution of cases predominantly in the under one year age group may be due to an immature immune status (2). Some authors also link the increased rate of fungal infections to the living conditions of the animals (poor hygiene, low-quality food, crowding in groups, cats living outdoors, gestation, immunosuppressive drug therapy, stress from various sources) (8, 19). Microtrauma, especially in cats that are exposed to bites and scratches from other cats (such as in cats in shelters), is a factor favouring infection with *Microsporum* spp. (18).

Animals without dermatological lesions are considered potential reservoirs of dermatophytes, with numerous studies demonstrating high prevalences (28.2% to 53.6%) among animals without dermatological signs specific to *Microsporum* spp. infection (6).

No association between the presence of *Microsporum* infection and the sex of animals was demonstrated in our study, similar to data reported in the literature (7).

Animal shelters meet a multitude of the predisposing factors that predispose cats to dermatophyte infection, i.e. predispose prospective owners considering adopting these animals from shelters to dermatophyte infection (18).

Conclusions

The overall prevalence of *Microsporum* infection in the stray cat population studied was 48%. No differences in prevalence were observed between animals of different sexes.

Animals in the under one year age group are more prone to *Microsporum* infection, with a significantly higher prevalence rate compared to the over one year age group-68% positive in the under one year age group, respectively 32% of positive cases were found in the over one year age group.

In terms of lesions, a 10% positive rate was observed in the group of animals without dermatological lesions compared to an 85% positive rate in the group of animals with lesions.

References

1. **Brilhante, R.S.N., Cavalcante, C.S.P., Soares-Junior, F.A., Cordeiro, R.A., Sidrim, J.J.C., Rocha, M.F.G.,** High rate of *Microsporum canis* feline and canine dermatophytosis in Northeast Brazil: Epidemiological and diagnostic features, Mycopathologia, 2003, 156, 4, 303-308.
2. **Guaguere, E., Prelaud, P.,** A practical guide to feline dermatology, Merial, 1999.
3. **Ilhan, Z., Karaca, M., Hakki Ekin, I., Solmaz, H.,** Detection of seasonal asymptomatic dermatophytes in Van cats, Brazilian Journal of Microbiology, 2016, 47, 225-230.
4. **Mancianti, F., Nardoni, S., Cecchi, S., Corazza, M., Taccini, F.,** Dermatophytes isolated from symptomatic dogs and cats in Tuscany, Italy during a 15-year-period, Mycopathologia, 2002, 156, 13-8.
5. **Mattei, A.S., Beber, M.A., Madrid, I.M.,** Dermatophytosis in Small Animals, Microbiological Infectious Diseases, 2014, 2, 3, 1-6.
6. **Mederle, N., Dărăbuș, Gh., Nichita, I., Morariu, S., Suici, T., Kumbakisaka, S., Buzatu, R., Gartner, A.,** Identification of *Microsporum canis* in cutaneous lesions of cats from Timis County, Lucrări Științifice Medicină Veterinară Ion Ionescu de la Brad University of Agriculture and Veterinary Medicine, 2017, 60, 223-226.
7. **Miller, W.H., Griffin, C.E., Campbell, K.L.,** Small animal dermatology, 7th Edition, Ed. Elsevier, 2013.
8. **Minarikova, A., Hauptman, K., Jeklova, E., Knotek, Z., Jekl, V.,** Diseases in pet Guinea pigs: a retrospective study in 1000 animals, Veteterinary Records, 2015, 177, 1-9, 2015.
9. **Moretti, A., Agnetti, F., Mancianti, F., Nardoni, S., Righi, C., Moretta, I., Morganti, G., Papini, M.,** Dermatophytosis in animals: epidemiological, clinical and zoonotic aspects, Italian Journal of Dermatology and Venereology, 2013, 148, 563-72.
10. **Moriello, K.,** Feline Dermatophytosis- aspects pertinent to disease management in single and multiple cat situations, Journal of Feline Medicine and Surgery, 2014, 16, 419-431.
11. **Pinard, M., Chermette, R., Bussie´Ras, S.,** Diagnostic et prophylaxie des teignes des carnivores domestiques. Etude critique a` partir d'une enquete a` l'Ecole Nationale Ve´te´rinaire d'Alfort, Recueil de Medecine Veterinaire, 1987, 163, 1107-16.
12. **Pinter, Lj., Jurak, Z., Ukalovic, M.M., Susic, V.,** Epidemiological and clinical features of dermatophytoses in dogs and cats in Croatia between 1990 and 1998, Veterinaski Arhiv,1999, 69, 5, 261-70.
13. **Richard, J.l., Bebey, M.C., Chermette, R., Pier, A.C., Hasegawa, A., Lund, A., Bratberg, A.M., Padhye, A.A., Connole, M.D.,** Advances in veterinary mycology, Journal of Medical and Veterinary Mycology, 1994, 32, 169-187.

14. **Sacheli, R., Adjetey, C., Darfouf, R., Harag, S., Huynen, P., Meex, C., Descy, J., Melin, P., Arrese, J., Hayette, M.-P.,** A one-year survey of *Microsporum audouinii* infections in Belgium: epidemiological and genotypic characterization, Parasitology, 2015, 22, 3, 285.e9-1.
15. **Sakuragi, Y., Sawada, Y., Hara, Y., Ohmori, S., Omoto, D., Haruyama, S., Yoshioka, M., Nishio, D., Nakamura, M.,** Increased circulating Th17 cell in a patient with tinea capitis caused by *Microsporum* canis, International Journal of Molecular Sciences, 2016, 65, 215-216.
16. **Shokri, H., Khosravi, A.R.,** An epidemiological study of animals dermatomycoses in Iran, Journal de Mycologie Médicale, 2016, 26, 170-177.
17. **Starkey, R.S.,** Dermatophytosis, Clinical Veterinary Advisor, 2013, 703-705.
18. **Taplin, D., Zaias, N., Rebell, G., Blank, H.,** Isolation and recognition of dermatophytes on a new medium (DTM), Archives of Dermatological Research, 1969, 99, 2, 203-9.
19. **Thomasa, J.E., Staubusb, L., Goolsbya, J.L., Reicharda, V.M.,** Ectoparasites of free-roaming domestic cats in the central United States, Veterinary Parasitology, 2016, 228, 17-22.
20. **Van Cutsem, J., Rochette, F.,** Mycoses in domestic animals, Janssen Research Foundation, Beerse, Belgium, 1991.

RESEARCH ON THE AEROBIC MICROBIOTA ISOLATED FROM THE HORSE NASAL CAVITY

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Summary

The research aimed to evaluate the aerobic microbiota in the nasal passages of horses with clinical signs and clinically healthy horses. The samples were collected from a number of 30 horses with clinical signs and clinically healthy horses, from the western part of Romania. The samples came from *Nonius, Romanian Half-Heavy, Ardennais, Purebred Arabian* and half-breed horses between the ages of 5 months and 9 years. From the 30 collected samples, 49 bacterial strains were isolated and identified, distributed as follows: *Streptococcus equi* subsp *zooepidemicus* 21 strains, *Staphylococcus* sp. 23 strains, *Pasteurella* sp 2 strains, *Proteus* and *E. coli* one strain each. The staphylococcal strains isolated were 100% sensitive to the following antibiotics: ampicillin/sulbactam, novobiocin, ceftriaxone, cefoxitin, cefaclor, rifampicin and ciprofloxacin, and to tetracycline and doxycycline the staphylococcal strains showed increased resistance. *Streptococcus equi* subsp *zooepidemicus* strains were 100% sensitive to the following antibiotics: ampicillin/sulbactam, ceftriaxone, cefoxitin, cefaclor, ciprofloxacin, rifampicin, methicillin, novobiocin, vancomycin and resistant to lincomycin and polymyxin.

Keywords: *Streptococcus equi subsp zooepidemicus*, *Staphylococcus aureus.*

Bacteria located in the nasal cavity and guttural pouches of infected animals play an important role in spreading the infection (1, 3, 11, 12, 15).

Regardless of the disease in question, the success of preventive measures depends on a correct assessment of the epidemiological context of the disease, especially its geographical distribution, incidence and prevalence (8, 20).

The bacterium *Streptococcus equi subsp. equi* is the causative agent of the highly contagious respiratory disease in horses called gurma (6, 12, 13). *Streptococcus equi subsp. zooepidemicus (S. zooepidemicus*) is considered an opportunistic commensal of the equine upper respiratory tract but is also known to cause disease in several animal species and occasionally in humans (2, 4, 14, 16).

Determining the genetic relationships between different strains of *S. equi* and *S. zooepidemicus* is important in epidemiological investigations of outbreaks in both horses and humans (3).

Human and equine isolates of *S. zooepidemicus* have revealed zoonotic transmission of certain strains of *S. zooepidemicus* from healthy horses that have caused severe disease in humans.

The speed of diagnosis is of great importance to prevent the spread of the disease, as it has high morbidity (7).

Materials and methods

The samples were collected from a number of 30 horses with clinical signs and clinically healthy horses, from the western part of Romania. Suspicion of disease in the respiratory system was signaled by the detection of the following clinical signs: fever, cough, nasal secretions, enlarged lymph nodes, change in respiratory rate and lack of appetite.

After sampling, the samples were kept in the refrigerator, and later they were transported under optimal conditions to the Bacterial Infectious Diseases laboratory within FMV Timișoara.

Cultivation and identification of bacterial species were carried out according to the standard methodology.

The collected samples were inoculated in order to carry out the cultural examination on 5% calf blood agar, respectively BHI (Brain Heart Infusion) agar. All cultures were incubated at 37°C for 24-48 hours.

After isolation in pure culture, the identification of the species, respectively the typing of the etiological agent, was carried out. Identification of the isolated bacteria was carried out based on morphological characteristics, cultural characters, and biochemical properties.

The definitive identification of suspected etiological agents was made with the help of diagnostic kits: API 20 E (API – Analytical Profile Index) - for the identification of pathogens from the Enterobacteriaceae family, API Staph – for the identification of staphylococci and API 20 Strep for the identification of streptococci (23).

The hemolytic activity of the isolated staphylococcus strains was shown on 5% calf blood agar. To highlight the bound coagulase, the rapid Prolex Staph Latex Kit produced by Pro-Lab Diagnostics was used.

The determination of antibiotic resistance of the isolated strains was carried out by the disc diffusimetric method (Kirby - Bauer method), using the Muller – Hinton medium and biodiscs impregnated with antibiotics, provided by the producing companies, respectively the following antimicrobial substances: Ampicillin/sulbactam (SAM), Cefaclor (CEC), Cefoxitin (FOX), Ciprofloxacin (CIP), Doxycycline (DO), Erythromycin (E), Gentamicin (CN), Kanamycin (K), Tetracycline (TE), Methicillin (ME), Novobiocin (NV), Polymyxin (PB), Rifampicin (RA), Vancomycin (VA).

The interpretation of the results was done according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) document. The diameter of the zone of inhibition was measured with a ruler and expressed in millimeters. Depending on the diameter read, the results were classified into: sensitive, intermediate and resistant (22).

Results and discussions

Of the 30 samples, 20 samples were collected from apparently clinically healthy horses present in the Giulvăz slaughterhouse, the rest of the samples being collected from horses with clinical signs from the localities: Ciacova, Cebza, Petroman and Izvin.

The samples came from *Nonius, Romanian Half-Heavy, Ardenez, Purebred Arabian*, and half-breed horses between the ages of 5 months and 9 years.

After 24 hours, Gram-stained smears were performed from the primary culture obtained, then passages on the selective medium for Gram-negative bacteria, blood agar and Chapman's Medium for Gram-positive bacteria.

The media were incubated under aerobic conditions at 37°C to be examined after 24 hours. Colonies that developed on Levine's medium were plated on MIU medium and TSI agar to highlight biochemical characteristics.

Colonies obtained on Chapman medium and 5% blood agar were Gram-stained and examined under a microscope. On the basis of the morphological aspect and minimal biochemical characters (catalase test and mannitol fermentation) it was possible to characterize and identify the isolated strains.

The final identification of the strains of bacteria belonging to the *Enterobacteriaceae* family was possible with the help of the API 20E kit, the strains of *Staphylococcus spp*. with the help of the API Staph kit, and the strains of *Streptococcus spp*. with the help of the API Strep 20 kit.

Table 1

Distribution of bacterial strains isolated from the nasal cavity of horses

The pathogen	Number of strains	%
Streptococcus equi subsp equi	1	2.0
Streptococcus equi subsp zooepidemicus	21	42.9
S. aureus	12	24.5
S. hyicus	3	6.1
S. epidermidis	6	12.2
S. sciuri	2	4.1
Proteus	1	2.0
Pasteurella sp.	2	4.1
E coli	1	2.0
Total	**49**	**100**

From the 30 collected samples, 49 bacterial strains were isolated and identified according to Table 1, being distributed as follows: *Streptococcus equi subsp equi* one strain, *Streptococcus equi subsp zooepidemicus* 21 strains, *Staphylococcus sp.* 23 strains, *Pasteurella sp* 2 strains, *Proteus* and *E. coli* one strain at a time.

The 49 isolated strains were distributed as follows: *Streptococcus equi*

subsp zooepidemicus 42.9%, *S. aureus* 24.5%, *S. epidermidis* 12.2%, *S. hycus* 6.1%, *S. sciuri* and *Pasteurella spp*., 4.1%, respectively *Streptococcus equi subsp equi*, *Proteus* and *E. coli* 2%, according to Fig. 1.

A single strain of *Streptococcus equi subsp equi* was isolated from the nasal cavity of a 9-month-old foal.

The *Proteus* strain was isolated from the nasal cavity of a 7-month-old foal belonging to the Izvin Stud.

All strains of *Staphylococcus spp*. isolates had characteristic behavior. By Gram staining, they were included in the group of Gram-positive bacteria, they showed the shape of cocci, grouped in the form of clusters (5, 6).

The isolated *Pasteurella* strains had biochemical properties similar to those described in the specialized literature (12, 16, 20, 21).

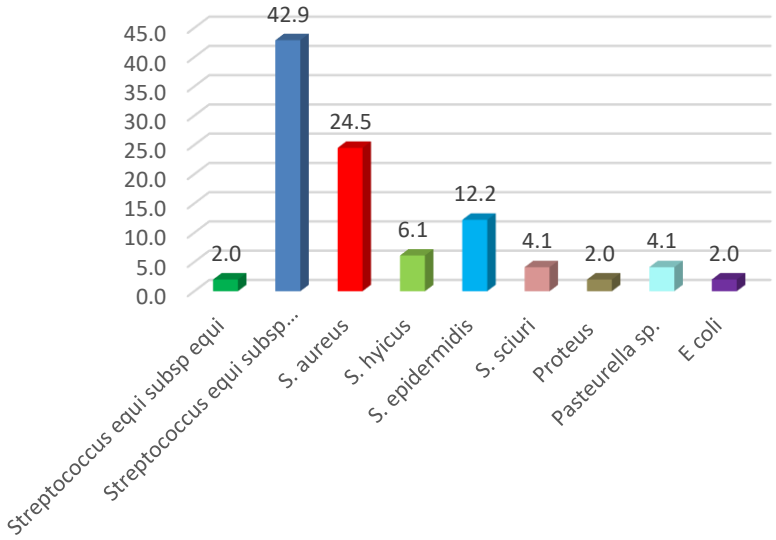

Fig. 1. Distribution of isolated strains from horse nasal swabs

Of the 23 strains of *Staphylococcus spp*. isolated from the nasal cavity of horses, 15 strains were coagulase-positive (*S. aureus* 12 strains and *S. hyicus* 3 strains) and 8 coagulase-negative strains (*S. epidermidis* 6 strains, *S. sciuri* 2 stems) according to Table 2. and Fig. 2.

Table 2

Distribution of staphylococci strains isolated from the nasal cavity of horses

Staphylococcus sp		**Number of strains**	**%**
Coagulase *positive* 15 strains	***S. aureus***	12	52.17
	S. hyicus	3	13.04
Coagulase *negative* 8 strains	***S. epidermidis***	6	26.09
	S. sciuri	2	8.70
Total		**23**	**100.00**

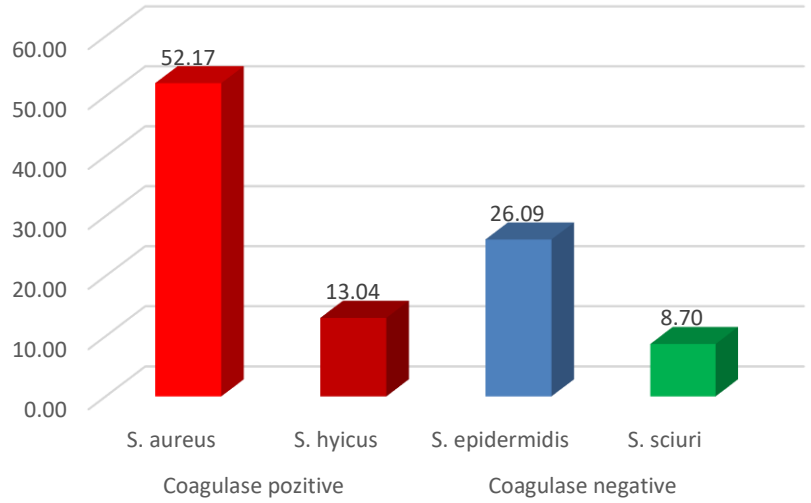

Fig. 2. Distribution of staphylococci strains isolated from the nasal cavity of horses

The hemolytic activity of staphylococcal strains was demonstrated on 5% blood agar.

With the help of the API Staph kit, the final identification of staphylococci strains was carried out. *S. aureus* strains had similar biochemical behavior which allowed their easy identification.

The highlighting of the bound coagulase (Clumping factor) was done with the help of the rapid kit Prolex Staph Latex Kit produced by the company Pro-Lab

Diagnostics.

The appearance of small clots indicated the presence of coagulase bound to 15 strains out of a total of 23 strains isolated from horse nasal cavities.

The results obtained from the antibiotic sensitivity testing of some strains of staphylococci isolated from the nasal cavity of horses are shown in table 3 and Fig. 3.

All staphylococcal strains isolated were 100% sensitive to the following antibiotics: ampicillin/sulbactam, novobiocin, ceftriaxone, cefoxitin, cefaclor, rifampicin and ciprofloxacin.

Staphylococcus strains showed increased resistance to tetracycline and doxycycline.

Table 3

Antibiotic susceptibility of staphylococci strains isolated from the nasal cavity of horses

Antimicrobial substance		Number of susceptible isolates			
		S. aureus	***S. hyicus***	***S. epidermidis***	***S. sciuri***
Ampicillin / sulbactam 30µg	SAM	12	3	6	2
Cefaclor 30µg	CEC	12	3	6	2
Cefoxitin 10µg	FOX	12	3	6	2
Ceftriaxone 30µg	CRO	12	3	6	2
Ciprofloxacin 30µg	CIP	12	3	6	2
Doxycycline 30µg	DO	7	2	1	2
Erythromycin 15µg	E	6	2	1	2
Gentamicin 10µg	CN	8	2	6	2
Kanamycin 30µg	K	6	1	5	2
Lincomycin 30µg	L	5	2	2	2
Methicillin 30µg	ME	9	3	5	2
Novobiocin 30µg	NV	12	3	6	2
Polymyxin 50 IU	PB	0	0	2	0
Rifampicin 30µg	RA	12	3	6	2
Tetracycline 30µg	TE	7	2	2	2
Vancomycin 30µg	VA	12	3	6	2

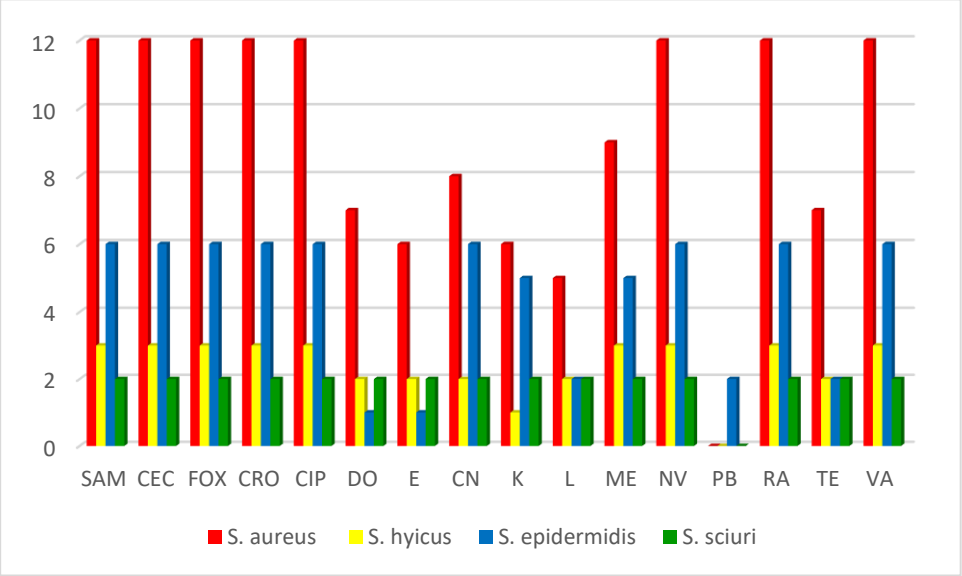

Fig. 3. Antibiotic susceptibility of staphylococci strains isolated from the nasal cavity of horses

Bacteriological examination of the samples collected from the nasal cavity of horses highlighted the presence of cocci chains characteristic of *Streptococcus spp.*, and later through conventional bacterial culture, they were isolated and identified.

On 5% blood agar, small, round, mucoid colonies with regular margins were observed, surrounded by an area of clear hemolysis (β - hemolytic streptococcus).

The identification of *Streptococcus spp* was first based on the evidence of its hemolytic capacity and its typical Lancefield group C antigens by a rapid latex agglutination test.

Highlighting the biochemical properties for the final identification of streptococci species was carried out using the API Strep 20 system.

Highlighting the biochemical properties, using the API 20 Strep system, in the case of *Streptococcus zooepidemicus* strains highlighted the fermentation of sorbitol and lactose, and in the case of the *Streptococcus equi* strain, it did not ferment sorbitol, lactose, and trehalose.

The results obtained are similar to those presented in the specialized literature (9, 17, 18, 19).

Table 4

Susceptibility of *Streptococcus zooepidemicus* strains (n=21) isolated from the nasal cavity of horses

Antimicrobial substance		Number of susceptible strains	% of sensitive strains
Ampicillin / sulbactam 30µg	SAM	21	100.0
Cefaclor 30µg	CEC	21	100.0
Cefoxitin 10µg	FOX	21	100.0
Ceftriaxone 30µg	CRO	21	100.0
Ciprofloxacin 30µg	CIP	21	100.0
Doxycycline 30µg	DO	14	66.7
Erythromycin 15µg	E	12	57.1
Gentamicin 10µg	CN	10	47.6
Kanamycin 30µg	K	19	90.5
Lincomycin 30µg	L	9	42.9
Methicillin 30µg	ME	21	100.0
Novobiocin 30µg	NV	21	100.0
Polymyxin 50 IU	PB	1	4.8
Rifampicin 30µg	RA	21	100.0
Tetracycline 30µg	TE	17	81.0
Vancomycin 30µg	VA	21	100.0

Increased sensitivity of *Streptococcus zooepidemicus* strains has been shown to the following antibiotics ampicillin/sulbactam, ceftriaxone, cefoxitin, cefaclor, ciprofloxacin, rifampicin, methicillin, novobiocin, and vancomycin.

Streptococcus zooepidemicus strains showed increased resistance to lincomycin and polymyxin (Table 4, Fig. 4.).

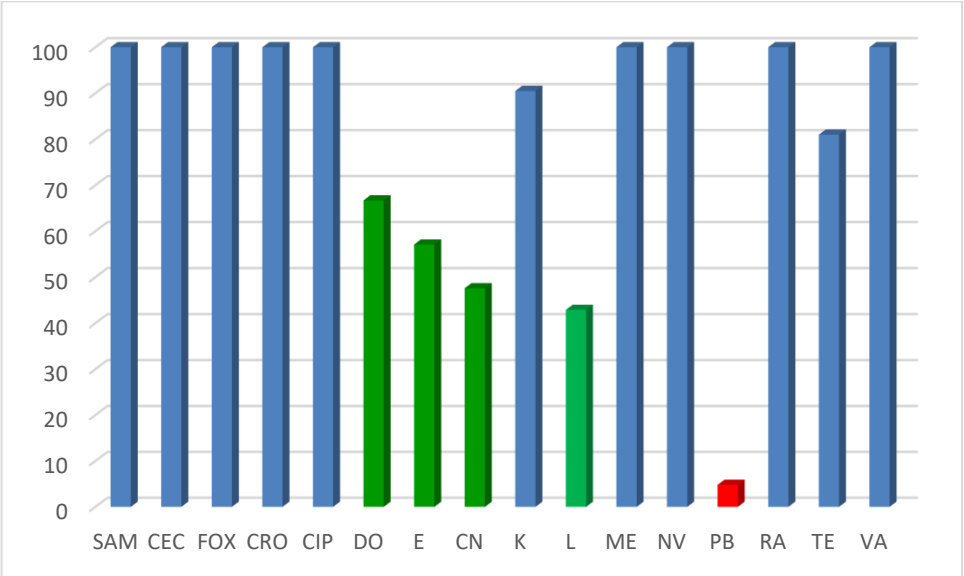

Fig. 4. Susceptibility of *Streptococcus zooepidemicus* strains (n=21) isolated from the nasal cavity of horses

Some authors recommend for the differentiation of streptococci, testing the fermentative capacity by inoculating it in broth containing ribose, sorbitol, trehalose and lactose, respectively (10, 18). Differentiating *Streptococcus equi* from other group C strains (especially *Streptococcus zooepidemicus*) is important because *Streptococcus equi* infections are highly contagious.

Therefore, rapid identification of *Streptococcus equi* is a high priority for the clinician.

Identification of asymptomatic carriers falls under offensive and defensive prophylaxis. This aspect of prophylaxis is all the more important as it appears that more than 75% of strangle outbreaks result in one or more asymptomatic carriers.

The rapid test provides a valuable aid in the identification of group C beta-hemolytic streptococci in an efficient and cost-competitive manner.

In this case, the recognition of beta-hemolytic streptococci is sufficient evidence to start treatment.

Anaerobic incubation is sometimes used for culture from nasal swabs to limit the growth of many contaminating bacteria (18).

Bacteria located in the nasal cavity and guttural pouches of infected animals play an important role in spreading the infection.

The international transport of horses, competitions, races and trade in horses could promote the spread of pathogenic strains of staphylococci and streptococci, both to other animals and to humans.

Conclusions

From the 30 samples collected from the nasal cavity of horses, the following pathogens were isolated: *Streptococcus equi subsp zooepidemicus* 42.9%, *S. aureus* 24.5%, *S. epidermidis* 12.2%, *S. hycus* 6.1 %, *S. sciuri* and *Pasteurella spp*., 4.1%, *Streptococcus equi subsp equi, Proteus* and *E. coli* 2%.

The staphylococcal strains isolated were 100% sensitive to the following antibiotics: ampicillin/sulbactam, novobiocin, ceftriaxone, cefoxitin, cefaclor, rifampicin and ciprofloxacin, and to tetracycline and doxycycline the staphylococcal strains showed increased resistance.

Streptococcus zooepidemicus strains were 100% sensitive to the following antibiotics ampicillin/sulbactam, ceftriaxone, cefoxitin, cefaclor, ciprofloxacin, rifampicin, methicillin, novobiocin, vancomycin and resistant to lincomycin and polymyxin.

It is recommended to identify pathogenic bacterial species in the nasal cavity of horses, with the help of rapid kits, to reduce the risk of transmission of diseases from one herd to another, by means of asymptomatic carriers.

References

1. **Beste, K.J., Lawhon, S.D., Chamoun-Emanuelli, A.M.,** Culture independent and dependent evaluation of the equine paranasal sinus microbiota in health and disease, Equine Veterinary Journal, 2020, 52, 455-463.
2. **Boyle, A.G., Rankin, S.C., Duffee, L., Boston, R.C., Wheeler-Aceto, H.,** *Streptococcus equi* detection polymerase chain reaction assay for equine nasopharyngeal and guttural pouch wash samples, Journal of Veterinary Internal Medicine, 2016, 30, 1, 276-281.
3. **Boyle, A.G., Stefanovski, D., Rankin, S.C.,** Comparison of nasopharyngeal and guttural pouch specimens to determine the optimal sampling site to detect *Streptococcus equi* subsp. *equi* carriers by DNA amplification, BMC Veterinary Research, 2017, 13, 75.
4. **Boyle, A.G., Timoney, J.F., Newton, J.R., Hines, M.T., Waller, A.S., Buchanan, B.R.,** *Streptococcus equi* infections in horses: guidelines for treatment, control, and prevention of strangles — revised consensus statement, Journal of Veterinary Internal Medicine, 2018, 32, 2, 633-647.
5. **Brinda, M., Herman, V., Fodor, I.,** Phenotypic characterization of coagulase-negative staphylococci isolated from mastitic milk in cows, Lucrări Ştiinţifice Medicină Veterinară Timişoara, 2010, XLIII, 1, 43, 97-101.
6. **Bucur, I., Pascu, C., Iancu, I., Herman, V., Degi, J., Cătană, N.,** Considerations regarding the methicillin resistance of staphylococci isolated from animals, Lucrări Ştiinţifice Medicină Veterinară Timişoara, 2018, LI, 4, 11-15.
7. **Cătană, N.,** Boli produse de germeni din genurile *Staphylococccus* și

Streptococcus. în Radu Moga Mânzat (coord.), Boli infecţioase ale animalelor, Ed. Brumar, Timisoara, 2001.

8. **Cordoni, G., Williams, A., Duram, A., Florio, D., Zanoni, R.G., Ragione, R.L.,** Rapid diagnosis of strangles (*Streptococcus equi* subspecies *equi*) using PCR, Research in Veterinary Science, 2015**,** 102, 162-166.
9. **Costinar, L., Herman, V., Pitoiu, E., Iancu, I., Degi, J., Hulea, A., Pascu, C.,** Boar semen contamination: Identification of Gram-negative bacteria and antimicrobial resistance profile, Animals, 2022, 12, 1, 43.
10. **Cvetojević, D., Radanović, O., Milićević, V., Jezdimirović, N., Kureljušić, B.,** Polyarthritis in goat-kids caused by streptococcus equi subspecies zooepidemicus, Acta Veterinaria-Beograd, 2017, 67, 3, 432-440.
11. **Dauvillier, J., Woort, F., Erck-Westergren, E.,** Fungi in respiratory samples of horses with inflammatory airway disease, Journal of Veterinary Internal Medicine, 2018, 33, 2, 968-975.
12. **Fodor, I.,** Observations upon the development of a Pseudomonosis episode in a broiler farm, Lucrări Ştiinţifice Medicină Veterinară Timişoara, 2007, XL, 375-378.
13. **Gomez, D.E., Arroyo, L.G., Lillie, B., Weese, J.S.,** Nasal bacterial microbiota during an outbreak of equine herpesvirus 1 at a farm in southern Ontario, Canadian Journal of Veterinary Research, 2021, 85, 3-11.
14. **Kim, J.W., Jung, J.Y., Lee, K., Lee, H., Kim, H.Y., Yoon, S.S., So, B.J., Choi, E.,** A case of *Streptococcus equi* subspecies *zooepidemicus* infection in a thoroughbred horse, Journal of Comparative Pathology, 2018, 158, 137.
15. **Laing, G., Christley, R., Stringer, A., Aklilu, N., Ashine, T., Newton, R., Radford, A., Pinchbeck, G.,** Respiratory disease and sero-epidemiology of respiratory pathogens in the working horses of Ethiopia, Equine Veterinary Journal, 2018, 50, 6, 793-799.
16. **Libardoni, F., Machado, G., Gressler, L.T., Kowalski, A.P., Diehl, G.H., Dos Santos, L.C., Corbellini, L.G., De Vargas, A.C.,** Prevalence of *Streptococcus equi* subsp. *Equi* in horse and associated risk factors in the State of Rio Grande do Sul, Brazil, Research in Veterinary Science, 2016, 104, 53-57.
17. **Pascu, C., Herman, V., Costinar, L., Iancu, I.,** Antimicrobial susceptibility of pathogenic bacteria isolated from swine lungs, Romanian Biotechnological Letters, 2019, 24, 3, 506-512.
18. **Pelkonen, S., Lindahl, S.B., Suomala, P., Karhukorpi, J., Vuorinen, S., Koivula, I., Väisänen, T., Pentikäinen, J., Autio, T., Tuuminen, T.,** Transmission of *Streptococcus equi* subspecies *zooepidemicus* infection from horses to humans, Emerging Infectious Diseases*,* 2013, 19, 7, 1041-1048.
19. **Rodríguez, N., Whitfield-Cargile, C.M., Chamoun-Emanuelli, A.M., Hildreth, E., Jordan, W., Coleman, M.C.,** Nasopharyngeal bacterial and fungal microbiota in normal horses and horses with nasopharyngeal cicatrix syndrome, Journal of Veterinary Internal Medicine, 2021**,** 35, 6, 2897-2911.
20. **Smith, F.L., Watson, J.L., Spier, S.J., Kilcoyne, I., Mapes, S., Sonder, C.,**

Pusterla, N., Frequency of shedding of respiratory pathogens in horses recently imported to the United States, Journal of Veterinary Internal Medicine, 2018, 32, 4, 1436-1441.

21. **Sweeney, C.R., Timoney, J.F., Newton, J.R., Hines, M.T.,** *Streptococcus equi* infections in horses: guidelines for treatment, control and prevention of strangles, Journal of Veterinary Internal Medicine, 2005, 19, 1, 123-134.
22. ***European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2021, Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1. Available: http:// www.eucast.org/
23. ***Biomerieux, API, Available at: www.biomerieux-usa.com/clinical/api

DETERMINATION METHODS OF CORTISOL LEVELS IN DOGS- OVERVIEW

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Summary

Cortisol is quantitatively the major glucocorticoid product of the adrenal cortex. Cortisol is often used as a stress indicator in animal behaviour research. This hormone can be measured using several different mediums like blood, saliva, urine, hair and even nail clippings. These parameters provide an acute, time-point measurement of cortisol concentration. When using invasive methods such as blood sampling, the measured cortisol concentration can be affected by several factors, such as circadian rhythms, handling, restraint, and degree of habituation or acclimation. In practical medicine, the aim of determining cortisol levels dictates the method used by the practician. In recent years, this hormone was up to debate whether measuring its levels can truly be an objective method of determining stress. Investigations in many species often rely exclusively on non-invasive sampling methods, because commonly applied analyses using plasma would be either not possible or simply inappropriate because their high invasiveness can cause stress responses. Initially principally measured in blood, cortisol measurement methods are now evolving towards lower invasiveness and allowing repeated measurements over time. In the case of small animals, especially dogs, in practical medicine, cortisol is often used as an indicator of hormonal diseases and salivary cortisol has been mainly used as a measure of the stress response, because saliva collection is less invasive than blood sampling, resting cortisol concentrations are routinely measured in dogs with chronic gastrointestinal signs to rule out hypoadrenocorticism.

Keywords: cortisol, stress, method, dog.

Initially principally measured in blood, cortisol measurement methods are now evolving towards lower invasiveness and allowing repeated measurements over time. Using non-invasive methods may be the key to measuring cortisol levels objectively, without external factors intervening in changing the cortisol level thus, having inconclusive results (7). Non-invasive measures of glucocorticoid (GC) hormones and their metabolites, particularly in feces and hair, are gaining popularity as wildlife management tools, but species-specific validations of these tools remain rare. Identification of severe stress in hospitalized veterinary patients may improve treatment outcomes and welfare (21).

Materials and methods

Subjects, articles, and selection criteria

Due to cortisol being a highly researched marker in the past decades, we compiled a list of key features we wanted to decrease the rate of the variability of the analyzed studies. Thus, a consensus was reached, for the selection of minimum criteria that had to be met by a certain study in order to be analyzed in this paper. In order to be analyzed, a study must be classified in the requirements described in Table 1.

Table 1

Article selection criteria

Years range	2007 - 2022	
Keywords	cortisol, method, detection, dog	
No. of samples	≥ 10 individuals ≤ 5 if comparing different detection methods 5-10 – without significant differences between methods and articles not included in statistical analysis	
No. of refferences in the article	≥ 25	
TOTAL ARTICLES SELECTED	***ISI journals***	***BDI/proceedings journals***
350	**70**	**280**

In order to be able to easily classify the articles, a list has been made in excel that specifically describes each searched category, respectively the numerical classification of the items under analysis. This is described in Table 2, Figures 1 and 2.

Table 2

Articles selected for analysis

No. of individuals used in the study	1-10	11-15	16-30	31-40	>40
No. of articles	253	55	23	3	6
No of articles meeting the criteria	128	12	19	3	3

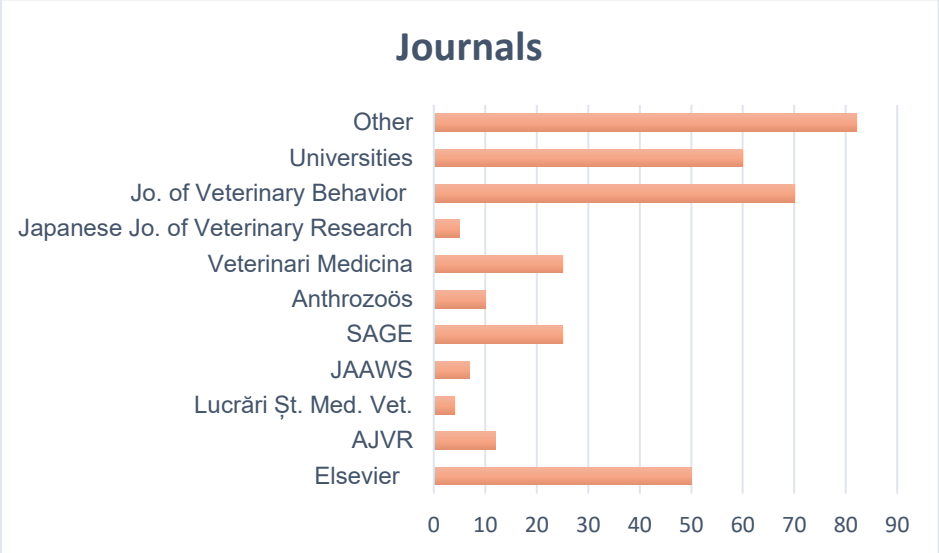

Fig. 1. The journals from where the articles were selected

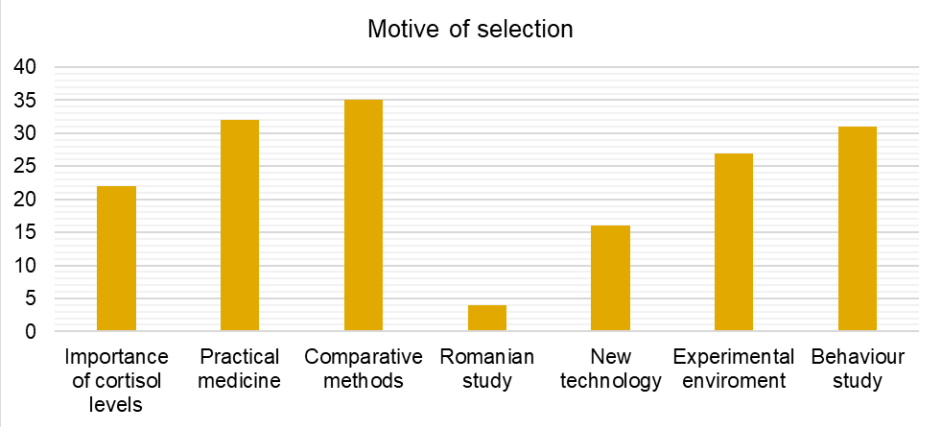

Fig. 2. Number of articles and the selection criteria

Results and discussions

By submitting for analysis all 350 articles found in the period 2007-2022, it was found that 156 fall within the minimum requirements and 11 were selected to be subjected to analysis for objective reasons. Those classified as having a number of

subjects less than 5 were selected by the cortisol detection methods used in the experiment, i.e. the cases were reported in the veterinary clinics. Chronic stress was followed in patients who underwent surgical operations and the detection of cortisol levels was performed over a minimum period of 60 days, in terms of the smallest observation interval respective 180 days the highest interval (24). Although the 11 articles did not meet the minimum criteria, the authors consider it necessary to mention such cases that have practicability in veterinary clinics.

Analysis of detection methods

Regarding the analysis of the collected data, it was necessary to divide the methods into matrices subjected to analysis. A divided approach by matrices will be discussed next, and eventually, the cumulative analysis of the general data collected and the results will be made.

From the point of view of the analysis, the matrices are subdivided into the sampling method as follows: invasive and non-invasive. A classification of matrices was also made in terms of cortisol persistence in the substrate. Thus, the matrices will be discussed from this point of view as short-term cortisol in which saliva and blood fall, medium-term cortisol in which the faeces and urine are classified, and respectively long-term cortisol in the hair and nails (2, 4, 11, 16).

Invasive methods

Invasive methods are those methods of collecting samples, in which strict confinement of the animal is required, which may result in difficulties in collecting the matrix. For objective reasons, the authors decided to include saliva and blood in this category.

From 2007 to 2022 in terms of data collection, 75 articles on cortisol detection with blood as a substrate were analyzed. In contrast, in terms of saliva, the total number of articles studied was 50.

The articles were selected and subcategorized in the motivation of cortisol detection in the form of: stress, pathologies, pain, aggressiveness, human-animal relationship and detection methods.

For a good understanding, it was graphically represented, all the subcategories made in Fig. 3 and 4.

Noninvasive methods

Noninvasive methods are represented by all methods of collecting matrix that do not require a form of brutal confinement of the dog or those who do not acquire the approach of the animal. This is where the matrices were framed: urine, faeces, hair and nails. From 2007 to 2022, urine data were collected from 20 articles, for faeces 6, for nails also 6 articles were analyzed, respectively 10 for hair. Table 3 shows the dispersion over time of the articles taken into account on the matrix and the year in which the article was published. Fig. 5 shows the prevalence of publications following the peaks of the years studied.

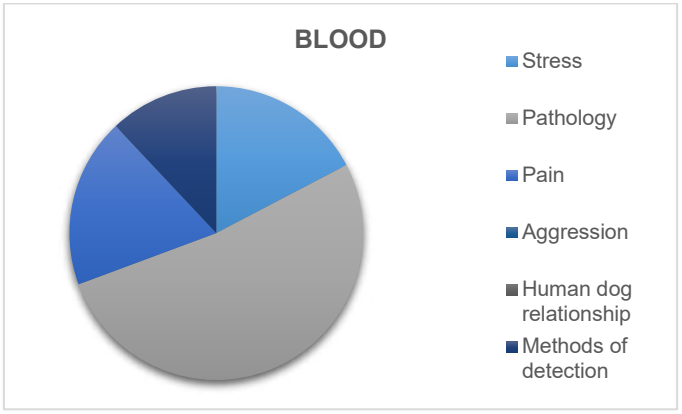

Fig. 3. Percentage distribution of the articles that used blood as matrix for cortisol assessment

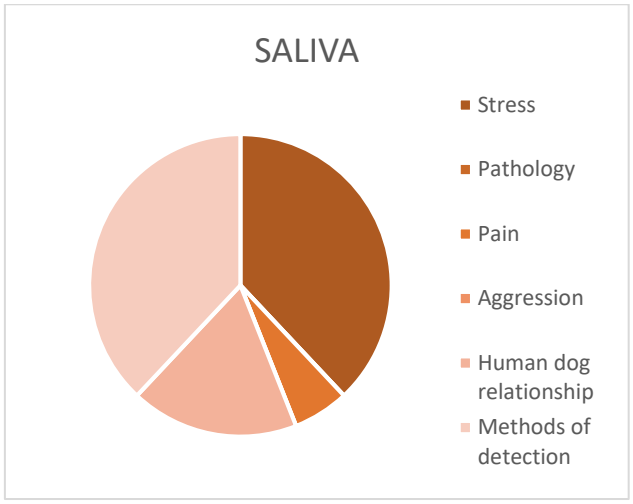

Fig. 4. Percentage distribution of the articles that used saliva as matrix for cortisol assessment

Table 3

Temporal dispersion of the articles – total number per year and total number per matrix

YEAR	METHOD OF SAMPLING BY MATRIX						
	Urine	Blood	Saliva	Feaces	Hair	Nail	Total
2007	3	5	2	0	0	0	10
2008	5	20	15	1	0	0	41
2009	0	12	5	0	0	0	17
2010	2	5	0	0	0	0	7
2011	1	6	3	0	0	0	10
2012	2	3	0	0	0	0	5
2013	1	5	0	1	0	0	7
2014	1	0	3	0	0	0	4
2015	2	6	0	2	0	0	10
2016	0	1	2	0	1	0	4
2017	0	4	2	0	0	0	6
2018	0	2	3	0	0	0	5
2019	0	0	3	0	1	1	5
2020	0	3	2	0	3	0	8
2021	0	1	5	0	2	3	11
2022	3	2	5	2	3	2	17
TOTAL	20	75	50	6	0	6	167

It can be noted that in the beginning the most used method of testing cortisol levels was by using blood as a matrix (11). From the studied articles, it is noted that, although blood is the most used substrate, the values reported by the authors have high variability, having as motivation the following arguments: the method of sampling is different from one article to another (e.g. jugular sampling, siphen sampling) the environment in which the study is carried out is most often different from one article to another or even during the experiment reported in a single article (14). The animal that is being tested, in this case, the dog, can be and is most often, of a different breed, different ages and even living in different types of environment. All this is being described as environmental variables (15, 18). Out of the total of 75 articles found, that use blood as a substrate for cortisol detection, 60% of the authors report that with this method it is difficult to appreciate cortisol as a relevant parameter for determining stress. This is supported by the fact that, when performing a blood sampling, the animal is subjected to some type of stress, and the serum cortisol levels might increase (20). Thus, we can say that the high variability found in the

literature and among the studies carried out between 2007-2022 (Fig. 5) is dependent on the individual for the most part and on the way of sampling, which can influence the result. From this point of view, blood is currently considered a method of detecting cortisol for revealing certain endocrine problems and not assessing stress. In a percentage of 30%, authors support the idea that although there is a risk of having uncertain results, if the animal, in this case, the dog, is desensitized to the sampling method before the beginning of the experiment, the inter- and intra-individual values have a much lower variability compared to the experiments in which the animal is subjected to contention without it being accustomed to the procedure (14, 22, 23, 25). The other authors cannot agree or oppose any of the theories, having experimental variability as motivation supports the idea that the environment, animal and man, involved in the experiment are the factors that determine the outcome of the experiment.

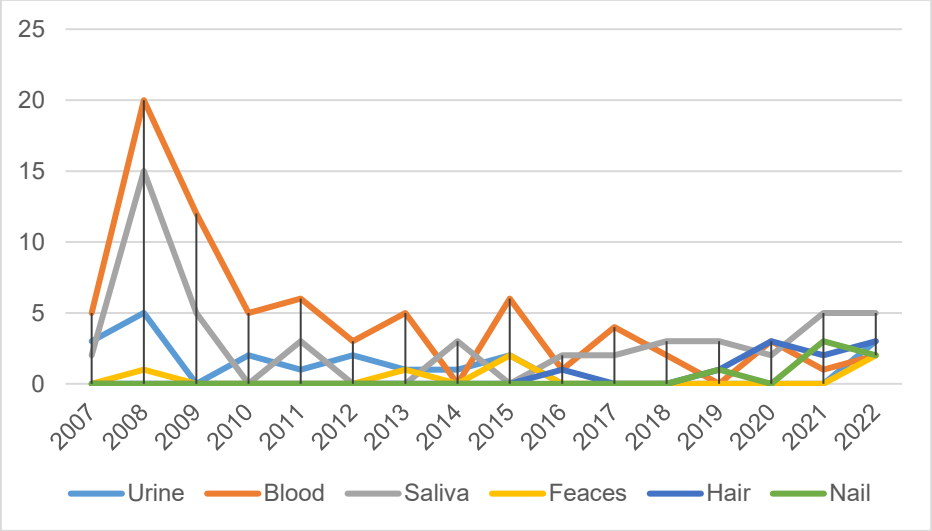

Fig. 5. Distribution of the articles by year and by matrix

When it comes to saliva sampling procedures, it is the workmanship that requires mention and not the results obtained. The problems most often related to the experiments that were mentioned in collecting samples of saliva were: uncooperative animals, the impossibility of collecting a sufficient amount of saliva to be able to use the ELISA kits (19) and the percentage of replicability of 60% intraexperimental (4, 6, 8, 9). From this point of view from the analyzed literature it is noted that saliva is considered to be a better matrix for the detection of cortisol compared to blood, but from an ethological point of view, it is also a method of assessment of acute stress (17, 19, 24).

When it comes to non-invasive methods, although the data collected are numerically inferior to the two previously discussed, uniformity can be observed in terms of the results obtained as well as some consensus on the theories launched by their authors(1, 3). It is observed that urine and faeces are two matrices used for cortisol detection in case of pathologies, and in practical medicine, they are used to assess stress in animals undergoing surgery to observe postoperatively, cortisol levels in relation to the medication received (2, 5). This is motivated by the persistence of cortisol in the two excretions of the organism over a longer period of time. Thus, cortisol levels can be correlated with a certain period of time during which the animal has undergone a certain treatment or intervention. It supports the idea that these two matrices are more often used in felines compared to the canine species, and thus the small number of found items using these two matrices can be justified (5, 7, 10, 21).

During the analysis, a massive trend of reorientation of studies based on cortisol determination was observed towards the use of matrices that do not require strict confinement methods. Although it is a relatively new trend among the research studies, a focus has been observed on the hair and nail type matrices. It seems that, from the analysis carried out, they could be the two best options at the moment to determine the cortisol values. These values are associated with the determination of chronic stress in animals, which is different from what was observed in classical matrices (1, 16). A major benefit is observed in terms of two branches of medicine, behavioural medicine and animal welfare. If until now, in terms of stress, the matrices used had the disadvantage that the determined values were correlated with the state of acute stress, by detecting the levels of cortisol in the hair, it is observed that the values correspond to chronic stress (12, 24). This is based on the principle of cortisol remanence and stability in the aforementioned matrices (hair and nail) during the assessment time interval, and even if the detected value is above the standard lower range for chronic stress, it can still be considered that cortisol levels correspond to chronic stress.

It is mentioned that, depending on the reason for the detection of cortisol levels, one can opt for a certain matrix, and the interpretation will be based on the principle of the method, but also on the purpose of using that matrix (1, 13). At present, the tendency to differentiate between matrices and the detected values is observed, thus eliminating, in most cases, the errors due to misinterpretation.

Conclusions

The most used method of measuring the levels of cortisol is blood**.** The key feature of this method is the type of restraint used is directly influencing the results. In practical medicine is commonly used for measuring basal cortisol in relation to different pathologies. In behavioural studies is commonly depicted as not the best method of determining stress in animals due to the nature of sampling. Hair collection is simple, inexpensive, and non-invasive, and can be performed easily and rapidly; thus, it appears to be a suitable method for determining the level of stress in dogs from shelters, abused dogs or dogs involved in different types of animal interactions. While acute stress disorder can be evaluated by means of measuring the cortisol concentration in blood and urine, chronic stress disorder can be detected by monitoring the cortisol concentration in fur or hair. Since it is a relatively new method, monitoring cortisol in hair or fur requires further research in order to definitively prove its efficacy, and possibly to determine reference range values for different breeds of dogs.

References

1. **Accorsi, P.A., Carloni, E., Valsecchi, P., Viggiani, R., Gamberoni, M., Tamanini, C., Seren, E.,** Cortisol determination in hair and faeces from domestic cats and dogs, General and Comparative Endocrinology, 2008, 155, 2, 398-402.
2. **Alberghina, D., Piccione, G., Pumilia, G., Gioè, M., Rizzo, M., Raffo, P., Panzera, M.,** Daily fluctuation of urine serotonin and cortisol in healthy shelter dogs and influence of intraspecific social exposure, Physiology & Behavior, 2019, 206, 1-6.
3. **Blackwell, E.J., Bodnariu, A., Tyson, J., Bradshaw, J.W.S., Casey, R.A.,** Rapid shaping of behaviour associated with high urinary cortisol in domestic dogs, Applied Animal Behaviour Science, 2010, 124, 3-4, 113-120.
4. **Carlone, B., Gazzano, A., Gutiérrez, J., Sighieri, C., Mariti, C.,** The effects of green odour on domestic dogs: A pilot study, Applied Animal Behaviour Science, 2018, 207, 73-78.
5. **Citron, L.E., Weinstein, N.M., Littman, M.P., Foster, J.D.,** Urine cortisol-creatinine and protein-creatinine ratios in urine samples from healthy dogs collected at home and in hospital, Journal of Veterinary Internal Medicine, 2020, 34, 2, 777-782.
6. **Colussi, A., Stefanon, B., Adorini, C., Sandri, M.,** Variations of salivary cortisol in dogs exposed to different cognitive and physical activities, Italian Journal of Animal Science, 2018, 17, 4, 1030-1037.
7. **Csoltova, E., Martineau, M., Boissy, A., Gilbert, C.,** Behavioral and physiological reactions in dogs to a veterinary examination: Owner-dog

interactions improve canine well-being, Physiology & Behavior, 2017, 177, 270-281.
8. **Dreschel, N.A, Granger, D.A.,** Methods of collection for salivary cortisol measurement in dogs, Hormones and Behavior, 2009, 55, 1, 163-168.
9. **Engerbretson, S.D.,** Effects of Multi-Day Music Enrichment on Salivary Cortisol and Cage Behavior in Shelter Dogs, 2018, Dissertations 3288.
10. **Fernandez, G.A., Boag, A., Gow, A.,** Evaluation of resting cortisol concentration testing in dogs with chronic gastrointestinal signs, Journal of Veterinary Internal Medicine, 2022, 36, 2, 525-531.
11. **Freeman, L.J., Rahmani, E.Y., Al-Haddad, M., Sherman, S., Chiorean, M.V., Selzer, D.J.,** Comparison of pain and postoperative stress in dogs undergoing natural orifice transluminal endoscopic surgery, laparoscopic, and open oophorectomy, Gastrointestinal Endoscopy, 2010, 72, 2, 373-380.
12. **Grigg, E.K., Nibblett, B.M., Robinson, J.Q., Smits, J.E.,** Evaluating pair versus solitary housing in kennelled domestic dogs (Canis familiaris) using behaviour and hair cortisol: a pilot study, Veterinary Record Open, 2017, 4, 1, e000193.
13. **Heimbürge, S., Kanitz, E., Otten, W.,** The use of hair cortisol for the assessment of stress in animals, General and Comparative Endocrinology, 2018, 270, 10-1.
14. **Hoglund, K., Lequarre, A.S., Ljungvall, I., Mc Entee, K., Merveille, A.-C., Wiberg, M., Gouni, V., Lundgren Willesen, J., Hanås, S., Wess, G., Mejer Sørensen, L., Tiret, L., Kierczak, M., Forsberg, S.K.G., Seppälä, E., Lindblad-Toh, K., Lohi, H., Chetboul, V., Fredholm, M., Häggström, J.,** Effect of Breed on Plasma Endothelin-1 Concentration, Plasma Renin Activity, and Serum Cortisol Concentration in Healthy Dogs, Journal of Veterinary Internal Medicine, 2016, 30, 566-573.
15. **Horváth, Z., Dóka, A., Miklósi, Á.,** Affiliative and disciplinary behavior of human handlers during play with their dog affects cortisol concentrations in opposite directions, Hormones and Behavior, 2008, 54, 1, 107-114.
16. **Jasmine, F., Antonella, C., Massimo, F., Alberto, P., Cristina, V.M.,** The usefulness of claws collected without invasiveness for cortisol and dehydroepiandrosterone (sulfate) monitoring in healthy newborn puppies after birth, Theriogenology, 2018, 122, 137-143.
17. **Kooriyama, T., Ogata, N.,** Salivary stress markers in dogs: Potential markers of acute stress, Research in Veterinary Science, 2021, 141, 48-55.
18. **Lee, S.K., Song, I.S., Park, S., Hong, Y., Chung, B.K.,** Changes in Serum Cortisol Concentration Due to Boarding Stress in Dogs, Journal of Veterinary Clinics, 2009, 27, 1, 35-41.
19. **Lucena, S., Coelho, A.V., Capela-Silva, F., Tvarijonaviciute, A., Lamy, E.,**The Effect of Breed, Gender, and Acid Stimulation in Dog Saliva Proteome, BioMed Research International, 2018, 1-12.

20. **Luño, I., Palacio, J., García-Belenguer, S., Rosado, B.,** Baseline and postprandial concentrations of cortisol and ghrelin in companion dogs with chronic stress-related behavioural problems: A preliminary study, Applied Animal Behaviour Science, 2019, 216, 45-51.
21. **Ottenheimer Carrier, L., Cyr, A., Anderson, R.E., Walsh, C.J.,** Exploring the dog park: Relationships between social behaviours, personality and cortisol in companion dogs, Applied Animal Behaviour Science, 2013, 146, 1-4, 96-106.
22. **Radisavljević, K., Vučinić, M., Becskei, Zs., Stanojković, A., Ostović, M.,** Comparison of stress level indicators in blood of free-roaming dogs after transportation and housing in the new environment, Journal of Applied Animal Research, 2017, 45, 1, 52-55.
23. **Rooney, N.J., Gaines, S.A., Bradshaw, J.W.S.,** Behavioural and glucocorticoid responses of dogs (Canis familiaris) to kennelling: Investigating mitigation of stress by prior habituation, Physiology & Behavior, 2007, 92, 5, 847-854.
24. **Srithunyarat, T., Hagman, R., Höglund, O.V., Stridsberg, M., Hanson, J., Lagerstedt, A.S., Pettersson, A.,** Catestatin, vasostatin, cortisol, and visual analog scale scoring for stress assessment in healthy dogs, Research in Veterinary Science, 2018, 117, 74-80.
25. **Steiss, J.E., Schaffer, C., Ahmad, H.A., Voith, V.L.,** Evaluation of plasma cortisol levels and behavior in dogs wearing bark control collars, Applied Animal Behaviour Science, 2007, 106, 1-3, 96-106.
26. **Wenger-Riggenbach, B., Boretti, F.S., Quante, S., Schellenberg, S., Reusch, C.E., Sieber-Ruckstuhl, N.S.,** Salivary Cortisol Concentrations in Healthy Dogs and Dogs with Hypercortisolism, Journal of Veterinary Internal Medicine, 2010, 24, 551-556.

OPTIMIZATION OF GENETIC DIAGNOSIS IN FELINE POLYCYSTIC KIDNEY DISEASE

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Summary

According to the data from the specialized literature, the usefulness of such tests in the certainty diagnosis of ADPKD is unanimously accepted, being the only ones capable of highlighting the diagnosis from birth (even intrauterine, although in practice such tests have not been performed) . Similar to the ultrasonographic examination, this investigative method is also extremely permissive in the sense that an extremely wide range of biological matrices can be analyzed, including gingival swabs, which makes the testing minimally invasive for the patient. Furthermore, sampling can be done by the owner himself, with minimal stress on the patient. Genetic testing was performed on 11 blood samples from individuals from susceptible breeds, following the protocol by genomic DNA extraction and point mutation identification by the Real Time PCR technique coupled with HRM. The results obtained show a uniform amplification of the analyzed samples, as well as the identification of individuals carrying the mutation, alongside individuals free of the mutation. According to the results obtained, the technique chosen for the extraction of the genetic material proved to be one suitable for the purpose, being able to extract the genetic material from samples considered to be difficult (whole blood).

Keywords: PCR, PKD, genetic diagnosis, cats.

According to the data from the specialized literature, the usefulness of such tests in the certainty diagnosis of ADPKD is unanimously accepted, being the only ones capable of highlighting the diagnosis from birth (even intrauterine, although in practice such tests have not been performed). Similar to the ultrasonographic examination, this investigative method is also extremely permissive in the sense that an extremely wide range of biological matrices can be analyzed, including gingival swabs, which makes the testing minimally invasive for the patient. Furthermore, sampling can be done by the owner himself, with minimal stress on the patient (2, 4, 7).

Although the genetic event responsible for the appearance of ADPKD benefits from a relatively generous number of detection methods, being a point mutation (SNP – single nucleotide polymorphism), however, the implementation of a diagnostic flow must be viewed with utmost seriousness so that at finally, the detection method used must comply with the specific requirements of laboratory diagnosis, especially those related to specificity, sensitivity, repeatability, reproducibility, but also aspects related to practicability (short time to obtain results, cost of analysis, etc.) (3, 8, 11).

Materials and methods

Genomic DNA extraction

The choice of nucleic acid extraction methodology must take into account a series of extremely important criteria, related to ensuring test performance but also to pecuniary aspects (the cost of this stage) and not least the possibility of standardization and ease of use. Thus, a commercial extraction kit was used for this step – QIAamp DNA mini kit (manufacturer Qiagen, Hilden, Germany), a kit that presents the following important features (1, 5, 6):

- Possibility of genomic DNA extraction from a wide variety of biological samples, including whole blood collected on EDTA, buccal swabs, tissues, biological or pathological fluids (13).
- Technology based on centrifugation and the use of purification columns with selective silicon membrane.
- Volume of biological sample sufficient for analysis (e.g. 200µl whole blood)
- The existence of proteinase K for the enzymatic digestion of the biological sample (15).
- The possibility of scaling the number of samples performed and possibly automation (9).

The work protocol used was the one recommended by the manufacturer, with some small differences depending on the type of sample used (whole blood or gingival swabs) (Table 1 and 2) (10).

Table 1

Genomic DNA extraction protocol from whole blood on EDTA

Proteinase K	**20µl**	**Mix by pipetting/light vortexing/inversion sealed tubes**
Sample	200µl	
Lysis buffer AL	200µl	
Incubation 15 minutes to 56°C with shaking the plate (1.000rpm)		
Spin type centrifugation for liquid collection		
Absolute ethanol	200µl	Mix by pipetting /energetic vortexing
Spin type centrifugation for liquid collection		
Sample transfer to purification colonies		Centrifugation at 12.000rpm for 1 minute. Collector tube change
Washing buffer AW1	600µl	

Incubate for 1 minute at room temperature. Centrifugation at 12,000 rpm for 1 minute. Change the collector tube		
Washing buffer AW2	600µl	Centrifugation at 12.000rpm for 1 minute. Collector tube change
Centrifugation at maximum speed for 2 minutes. Introduction of colony into the elution tube		
Elution buffer AE	150µl	Incubate for 1 minute at room temperature. Centrifuge at maximum speed for 1 minute

Table 2

Genomic DNA extraction protocol from gingival swabs

Sample sampling		**The sample is taken on the dedicated swabs. It is inserted into a 2ml tube**
Addition of phosphate buffer saline		Add 400 µl. Vortex vigorously and spin centrifuge to collect liquid
Proteinase K	20µl	Mix by pipetting. Incubate for 15 minutes at 56°C with shaking(1.000rpm)
Lysis buffer AL	400µl	
Absolute ethanol	200µl	Mix by vigorous pipetting/vortexing
Spin-type centrifugation for liquid collection		
Sample transfer to purification colonies		Centrifugation at 12.000 rpm for 1 minute. Change collector tube
Washing buffer AW1	600µl	
Incubate for 1 minute at room temperature. Centrifugation at 12.000 rpm for 1 minute. Change collector tube		
Washing buffer AW2	600µl	Centrifugation at 12.000 rpm for 1 minute. Change collector tube
Spin at maximum speed for 2 minutes. Introduction of colony into the elution tube		
Elution buffer AE	150µl	Incubate for 1 minute at room temperature. Centrifuge at maximum speed for 1 minute

Point mutation identification by the Real Time PCR technique coupled with HRM

The choice of primers for the amplification of the region of interest was carried out by using dedicated programs (BioEdit), a pair of primers being selected to amplify a fragment of 130 nucleotides (Fig. 1)

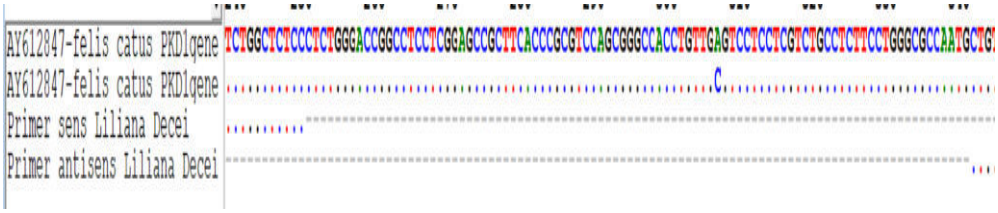

Fig. 1. Alignment of the primer sequences with the reference sequences of the PKD1 gene – it can be seen that the amplified fragment contains the point mutation of interest

The choice of amplification kit was based on the commercial availability of such a solution, and the Type-it HRM PCR Kit (Qiagen, Hilden, Germany) was selected. This solution is a dedicated one for DNA genotyping/typing in order to identify including point mutations. The thermal profile used was the one recommended by the manufacturer (Fig. 2), the composition of the reaction being presented in Table 3 (12).

Table 3

Amplification and detection protocol worksheet

Reagent	Volume/reaction	Final concentration
2x HRM PCR Master Mix	12.5µl	1X
Primer mix 10µM	1.75µl	0,7 µM primer 0,7 µM antisense primer
Ultrapure water	5.75µl	
DNA extracted	5µl	
Total volume	25µl	

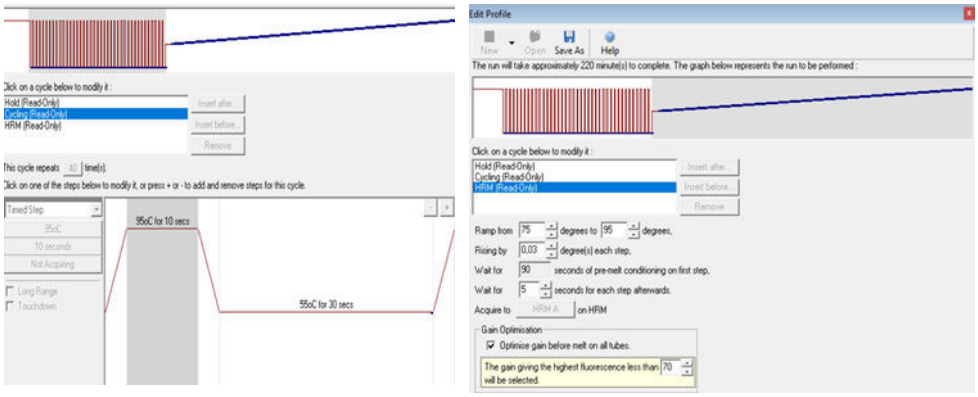

Fig. 2. Thermal profile used for analysis (RotorGene Q instrument)

Optimization and evaluation of assay results was performed by amplifying two previously diagnosed blood samples, one from a patient carrying the mutation and the other from a patient homozygous for the wild allele (Fig. 3, Fig. 4, Fig. 5).

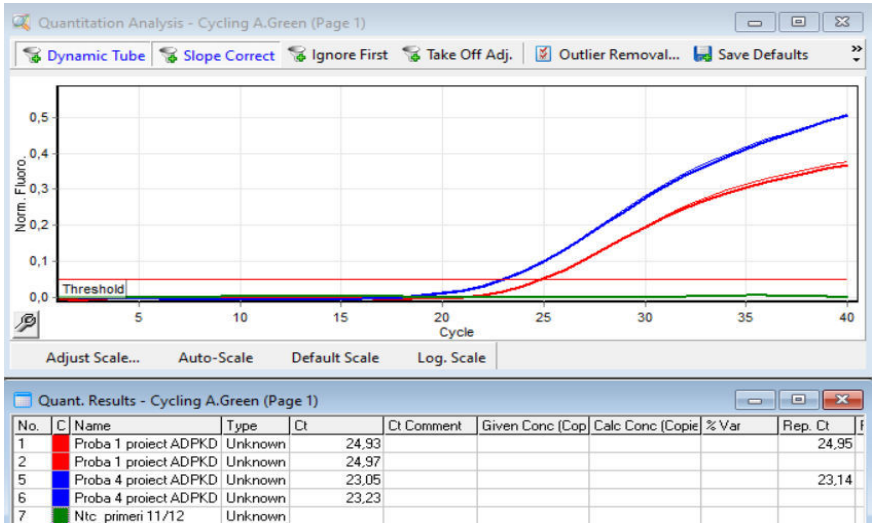

Fig. 3. Amplification curves obtained for reference samples (blue homozygous individual, red heterozygous individual). It can be seen that each sample was tested in duplicate

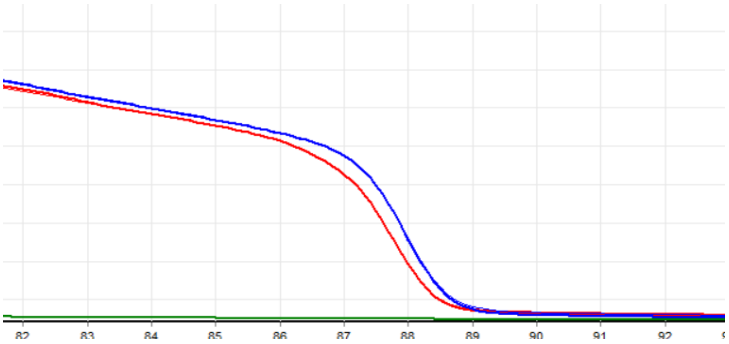

Fig. 4. The melting curves obtained for the reference samples (blue homozygous individual, red heterozygous individual). It can be seen that each sample was tested in duplicate

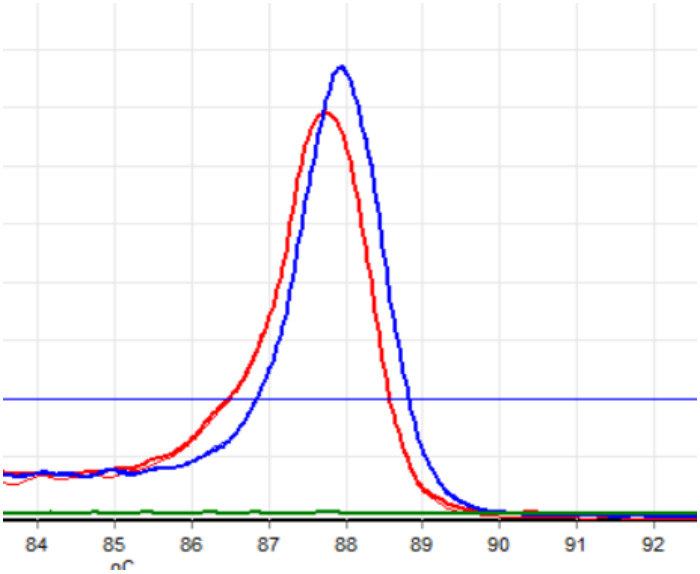

Fig. 5. Melting peaks obtained using the instrument's interpretation algorithm for reference samples (blue homozygous individual, red heterozygous individual). Each sample was tested in duplicate

Genetic testing was performed on 11 blood samples from individuals from susceptible breeds, according to Table 4. Following the protocol used to optimize the previously described method.

Table 4

Identification of samples analyzed by genetic technique

No. Crt.	Breed	Sex	Age	Ultrasound examination	Biochemical examination	Genetic testing result
1	Persian	M	3 years	**Kidney** – multiple microcysts bilaterally, diameter cca 5mm **Liver –** no changes **Pancreas –** no changes	-	+/-
2	Persian	F	8 years	Confirmed APKD	-	+/-
3	Persian	M	9 years	Free APKD	IRC stage IV	+/+
4	Persian	M	5 years	Free APKD	IRC stage IV	+/+
5	British Shorthair	M	15 years	-	uree – 130mg/dl creatinine – 12.9mg/dl ALT – 157U/L Blood sugar – 118mg/dl	+/+
6	Persian	F	3 years	Free APKD	-	+/+
7	Persian	M	5,5 years	Free APKD	-	+/+
8	Persian	M	2,5 years	-	-	+/+
9	Persian	M	7 years	Confirmed APKD	-	+/-
10	Persian	F	1.5 years	Free ADPKD	-	+/-
11	Persian	M	9 months	-	-	+/+

Results and discussions

The results obtained show a uniform amplification of the analyzed samples (Fig. 6), as well as the identification of individuals carrying the mutation, alongside individuals free of the mutation (Fig. 7). According to the results obtained, the

technique chosen for the extraction of the genetic material proved to be one suitable for the purpose, being able to extract the genetic material from samples considered to be difficult (whole blood) (16).

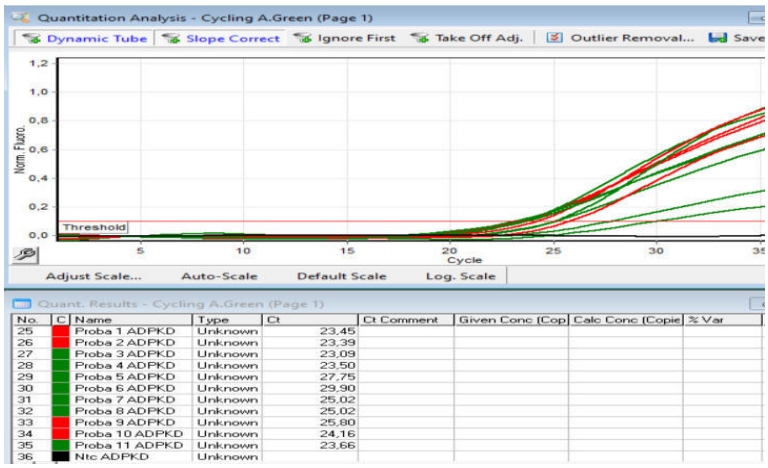

Fig. 6. Amplification curves obtained for the analyzed samples (red – mutation-carrying individuals, green – mutation-free individuals)

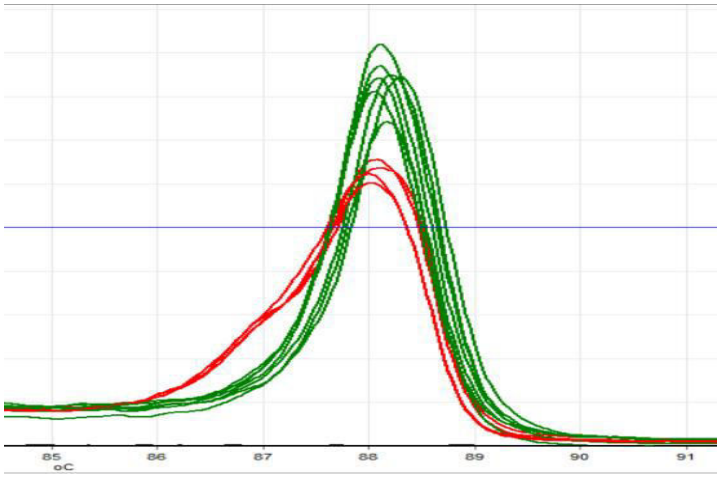

Fig. 7. The melting peaks obtained for the analyzed samples (red – mutation-carrying individuals, green – mutation-free individuals)

At the same time, through the possibility of scaling the number of samples that can be analyzed (individual samples as well as samples extracted on a format 96 plate), this protocol can be successfully used both in personalized diagnosis (several samples analyzed simultaneously) and also for a program of national screening (192 samples drawn simultaneously using a plate centrifuge). Through the composition of reagents, the technique is capable of extracting genomic DNA from a wide range of biological matrices, including gingival swabs, rectal swabs, etc., so that it becomes a minimally invasive diagnostic and self-screening method (possibility of sample collection by the owner, without additional stress to the animal).

Regarding the amplification/detection protocol, an adequate repeatability of it (as well as of the extraction step) can be found, so that the results obtained are certain, without ambiguities. And in this case the protocol used is simple to perform, even for an operator with average training in the field, so it can be easily implemented in laboratories with minimal equipment in the field of molecular biology (14, 17).

The results generated can clearly distinguish between a wild-type homozygous individual (well-individualized melting peak, generally with higher fluorescence amplitude/level) and a heterozygous individual (tendency to form a secondary melting peak, generally with a lower fluorescence) (18)

Last but not least, it should be mentioned that the results obtained for the reference samples were in full agreement with the results previously obtained using the gold standard (sequencing), a fact that strengthens the credibility of such a protocol and which, in addition, benefits from a rapidity and a clearly superior safety in operation – the results are obtained in approximately three hours from the reception of the sample, reaction in a single tube, with extremely reduced possibilities of contamination and generation of erroneous results.

The results obtained for the analyzed samples show a very good agreement with the ultrasonographic examination, except for one sample in which the result of the genetic test showed the existence of the mutation while the ultrasonographic examination failed to identify the cysts at the parenchymal level. However, upon a closer examination of the reason for the discrepancy, it is found that the investigated patient is young (1.5 years old), so it can be suspected that the macroscopic changes are not yet evident, so it is necessary to monitor the dynamics and identify the moment when they appear (19,20).

Consequently, a better structured diagnostic algorithm is required, namely genetic testing followed by ultrasound monitoring of the patient - or in other words, the genetic test is the test that gives the diagnosis, the ultrasound examination being the one that raises the suspicion and at the same time allows the dynamic monitoring of the severity of the condition (along with, of course, the paraclinical tests used to highlight the impairment of organ functionality).

Conclusions

The results obtained from the genetic testing of the patients show a uniform amplification of the analyzed samples, as well as the identification of individuals carrying the mutation, alongside individuals free of the mutation.

The technique chosen for the extraction of genetic material proved to be fit for purpose, being able to extract genetic material from samples considered to be difficult (whole blood).

The PCR diagnostic method through the addressed protocol can be successfully used both in personalized diagnosis (several samples analyzed simultaneously) and for a national screening program (192 samples extracted simultaneously using a plate centrifuge).

Through the composition of the reagents, the technique is able to extract genomic DNA from a wide range of biological matrices, including gingival swabs, rectal swabs, etc., so that it becomes a minimally invasive diagnostic and self-screening method.

Regarding the amplification/detection protocol, an adequate repeatability of it (as well as of the extraction step) can be found, so that the results obtained are certain, without ambiguities.

The results generated can clearly distinguish between a wild-type homozygous individual (well-individualized melting peak, generally with higher fluorescence amplitude/level) and a heterozygous individual (tendency to form a secondary melting peak, generally with a lower fluorescence).

The results obtained for the samples analyzed by molecular biology techniques show a very good agreement with the ultrasonographic examination.

These results support the formulation of a diagnostic algorithm structured in stages, by issuing the suspicion of polycystic kidney disease following the ultrasound examination and confirming the condition through genetic testing.

References

1. **Bilgen, N., Bişkin, T.M., Çinar, K.B., Isparta, S., Şen, Y., Akkurt, M.Y., Çildir, Ö.Ş., Bars, Z.,** Prevalence of PKD1 gene mutation in cats in Turkey and pathogenesis of feline polycystic kidney disease, Journal of Veterinary Diagnostic Investigation, 2020, 32, 4, 549.
2. **Bonazzi, M., Volta, A., Gnudi, G., Cozzi, M.C., Strillacci, M.G., Polli, M., Longeri, M., Manfredi, S., Bertoni, G.,** Comparison between ultrasound and genetic testing for the early diagnosis of polycystic kidney disease in Persian and Exotic Shorthair cats, Journal of Feline Medicine and Surgery, 2009, 11, 6, 430-434.
3. **Constantinescu (Preda), V., Popa, A., Codreanu, M.,** Dominante clinico patogenetice în nefropatiile polichistice la animalele de companie, Catlife, 2017, 6, 12.

4. **Criado-Fornelio, I., Buling, A. Barba-Carretero, J.C.,** Identification of feline polycystic kidney disease mutation using fret probes and melting curve analysis, Research in Veterinary Science, 2009, 86, 1, 88-90.
5. **Domanjko-Petric, A., Cernec, D., Corman, M.,** Polycystic kidney disease: a review and occurrence in Slovenia with comparison between ultrasound and genetic testing, Journal of Feline Medicine and Surgery, 2008, 10, 2, 115-9.
6. **Grahn, R.A., Biller, D.S., Young, A.E., Roe, B.A., Qin, B., Lyons, L.A.,** Genetic testing for feline polycystic kidney disease, Animal Genetics, 2004, 35, 6, 503-4.
7. **Helps, C.R., Tasker, S., Barr, F.J., Wills, S.J., Gruffydd-Jones, T.J.,** Detection of the single nucleotide polymorphism causing feline autosomal-dominant polycystic kidney disease in Persians from the UK using a novel real-time PCR assay, Molecular and Cellular Probes, 2007, 21, 1, 31-34.
8. **Lagerqvist, S.,** Polycystic kidney disease: genetik och sjukdomsförlopp hos katt, hund och människa. First cycle, G2E. Uppsala: SLU, Dept. of Animal Breeding and Genetics, 2009.
9. **Lee, Y.J., Chen, H.Y., Wong, M.L., Hsu, W.L.,** Molecular detection of autosomal-dominant feline polycystic kidney disease by multiplex amplification refractory mutation system polymerase chain reaction, Journal of Veterinary Diagnostic Investigation, 2010, 22, 3, 424-428.
10. **Malik, R.,** Genetic Diseases of Cats, Journal of Feline Medicine and Surgery, 2001, 3, 2, 109-113.
11. **Preda (Constantinescu), V., Turcitu, M., Codreanu, I., Popa (Cristian), A.M., Codreanu, M.,** The compared accuracy of the ultrasonographic examination and PCR technique in feline polycystic disease, Journal of Biotechnology, 2019, 305, 15, S81-S82.
12. **Ríos, N.B., Pérez, R.J., Sánchez, J.J., Hernández, C.R., Hernández, E., Galván, T.Mj.,** Diagnóstico genético de poliquistosis renal autosómica dominante mediante PCR múltiple [Genetic diagnosis of autosomal dominant polycystic kidney disease using multiplex-PCR], Nefrologia, 2009, 29, 4, 327-30.
13. **Son, A., Dhirapong, A., Dosev, K.D., Kennedy, M.I., Weiss, H.R., Hristova, R.K.,** Rapid and quantitative DNA analysis of genetic mutations for polycystic kidney disease (PKD) using magnetic/luminescent nanoparticles, Analytical & Bioanalytical Chemistry, 2008, 390, 7, 1829-35.
14. **Turcitu, M.A., Codreanu, M.D., Fernoagă, C., Codreanu, I., Cioranu, R.,** Implementation of molecular techniques for feline Autosomal dominant polycystic kidney disease (ADPKD), SYMPOSIUM „Contribution of the scientific research to veterinary medicine progress" book of abstracts, 2012, November 22-23.
15. **Turcitu, M.A., Coste, H., Alexandru, N., Codreanu, I., Codreanu, M.D., Olaru, I., Tamba, P., Zybaczynski, I.,** Evaluation of molecular biology

techniques for prionic protein genetic variability identification in sheep, Romanian Journal of Biochemistry, 2008, 45, 1.

16. **Wang, R., Moudgil, A., Jordan, S.C.,** Cystic diseases of the kidney, In Emery and Rimoin's: Principles and Practices of Medical Genetics, Vol. 2, 5th Ed., Philadelphia, PA, Churchill Livingstone, 2007.
17. **Welling L.W., Grantham J.J.,** Cystic and developmental diseases of the kidney - The kidney 4th ed (Brenner BM, and Rector FC, eds). Philadelphia: W.B. Saunders, 1991.
18. **Widmer, W.R., Mattoon, J.S., Vaden, S.,** Urinary tract, Small Animal Diagnostic Ultrasound (fourth edition), 2021.
19. **Wills, J.S., Barrett, L.E., Barr, J.F., Bradley, J.K., Helps, R.C., Cannon, J.M., Gruffydd-Jones, J.T.,** Evaluation of the repetability of ultrasound scanning for detection of feline polycystic kidney disease, Journal of Feline Medicine and Surgery, 2009, 11, 993-996.
20. **Yabuki, A., Mitani, S., Fujiki, M., Misumi, K., Endo, Y., Miyoshi, N., Yamato, O.,** Comparative study of chronic kidney disease in dogs and cats: Induction of myofibroblasts, Research in Veterinary Science, 2010, 88, 2, 294-299.

MANAGEMENT OF WHITE CLOVER PASTURE IMPACTING BOTH ANIMALS AND THE ECOSYSTEM

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Summary

The purpose of this paper was to analyze retrospectively the physiological requirements of white clover in order to have a culture that can be used for the benefits of clover in terms of nitrogen fixation in the soil and CO2 reduction in the air, responsible for the transformation of this into animal feed, thus having a double use. White clover swards are highly nutritious for both cattle and sheep which results in improved intakes and animal performance. Clover pastures fit well into forage or arable rotations and thus have a positive impact on soil fertility and structure. Ruminants have evolved to eat and utilize the nutrients in forage. However, methane emissions from livestock increase as forage quality reduce. Clover species are also important for providing honeybee forage and white clover is a major nectar flow for beekeepers. White clover can play an integral role in intensive grazing systems in terms of animal performance and herbage production. White clover (*Trifolium repens L.*), a species of plant in the Fabaceae family, is grown in diverse environments and is distributed in temperate and subtropical regions of the northern and southern hemispheres. Due to the high content of crude protein, white clover is widely grown as a fodder crop of legumes. White clover is often included in the perennial pastures of ryegrass (*Lolium perenne L.*). This mixed system is considered to be a high-quality feed, while improving the fertility of the soil, since legumes can fix atmospheric nitrogen with symbiotic bacteria. White clover is very demanding on the water factor. However, the content and persistence of white clover are often irregular both during years and over the years and between cultivated land on the same farm.
Keywords: white clover, animals, management, CO_2.

White clover (*Trifolium repens L.*), a species of plant in the *Fabaceae* family, is grown in diverse environments and is distributed in temperate and subtropical regions of the northern and southern hemispheres. It has been reported that white clover needs to be grown on the ground at a minimum of 20% to see the benefits of production. However, the content and persistence of white clover are often irregular both during years and over the years and between cultivated land on the same farm.

Materials and methods

In order to be able to determine the most favorable areas for the cultivation of clover, an analysis was made of the current data from the specialized literature

but also of the conditions in the areas where this crop is preferred among farmers, thus making a correlation between the general data but also the current conditions of clover cultivation. Few experimental results are known in the literature to elucidate the knowledge of the frost resistance potential of young clover plants (1, 4). There is a positive correlation between the growing season and latitude; a variety grown at a latitude greater than the place of origin shortens its growing season but produces a smaller amount of biomass (20, 18). A harmonious development of plants is ensured at a certain length period of the day (3, 8), characteristic of the latitude at which the variety was created (2, 10). Observations made in our country (9, 15, 19) have revealed that white clover sown in mixtures with perennial grasses blooms (17) later than red clover sown in pure culture (16). In our case, we conducted an experiment on a cattle farm by planting white clover.

The organization and conduct of experimental works consist of the application of the experimental technique specific to agricultural research.

The species and varieties of grasses and legumes of meadows taken under study were:

1. *Trifolium repens* L - **Apollo** variety
2. *Trifolium repens* L – **Rivendel** variety
3. *Lolium perenne* L – **Mathilde** variety

The location of the experience was carried out according to the method of the subdivided plots, in 3 repetitions (Table 1).

Table 1

Plot division by sowing method

R III	**b_1**	**b_2**	**b 3**	**b_4**	**b_5**	**b_6**	**b 1**	**b_2**	**b_3**	**b 4**	**b 5**	**b 6**	**b_1**	**b_2**	**b 3**	**b_4**	**b_5**	**b_6**
R II	b_1	b_2	b 3	b_4	b_5	b_6	b 1	b_2	b_3	b_4	b_5	b_6	b_1	b_2	b 3	b_4	b_5	b_6
R I	b_1	b_2	b 3	b_4	b_5	b_6	b 1	b_2	b_3	b_4	b_5	b_6	b_1	b_2	b 3	b_4	b_5	b_6
				a1						a_2							a_3	

(REPEATED GRAZING WITH GRAZING WITH MOWINGS) 2 UVM/HA 4 UVM/HA

Results and discussions

Nitrogen is "fixed" by a symbiotic relationship with *rhizobium* bacteria (3). The plant provides energy for bacteria (12), and bacteria convert atmospheric nitrogen into a form available to plants (15). A white clover culture will fix 100-150 kilograms of nitrogen per year, depending on the soil and growing conditions (7, 10, 19). At fertilizer nitrogen prices of 5 lei per kilogram at the purchase price analyzed at the time this study was carried out, the economic value of nitrogen fixation is

observed (5), which should be higher than the payment of expenses for seeds and cultivation. Thus, it is specified that a quantity of 70 kg of clover saves the cost of using 50 kg of NPK (6, 11, 12) with an approximate value of 250 lei / 50 kg (14). Due to the energy requirements involved in the process (21), ensuring optimal crop growth is fundamental to achieving high levels of nitrogen fixation, which in turn supports further crop growth. The importance of developing and streamlining biological nitrogen fixation systems becomes a priority for the agricultural economy of each country and for each farm, given that they ensure: profitability, energy efficiency, plant nutrition and human safety, the quality of the environment and, finally, sustainable agriculture. A negative issue associated with grass-only systems (8) is the need for significant amounts of chemical fertilizer containing nitrogen, such as urea. Nitrogen fertilizer inputs represent a large part of the total feed cost in forage-based livestock systems (15). Further, nitrogen fertilizers are a major source of nitrous oxide emissions in the feed production for herbivores and more efficient use of fertilizers is an important tool to mitigate nitrous oxide losses. Overseeding with clovers alone with no nitrogen fertilizer can result in calf body weight gains equal to those for annual ryegrass overseeded into bermudagrass and annually fertilized with 150 kg/ha N (15). If at the beginning of the study an amount of 12 l / cow/day was recorded, at the end of the study it was reached an average of 18 l /cow/day (4, 20).

The contribution of soil N uptake vs. fixation of atmospheric N to plant N acquisition was estimated 7 months after seeding with the N natural abundance technique.

$$\%Nfix=(\delta 15N_{plantref}-\delta 15N_{plantfix})/(\delta 15N_{plantref}-B) \times 100$$

Using clover to reduce the use of chemical nitrogen can reduce nitrous oxide emissions by up to 40% on a dairy farm due to reduced chemical N fertilizer application. The dilution method can provide an accurate time-integrated estimate of the amount of fixed N associated with the measurement of the grassy carpet. The accumulation rate of clover plants was the main determinant. The other two main factors influencing the calculated amount of N fixed are the total concentration of N in the clover plant and the proportion of total N derived from the fixation N2 in the soil. Honeybees can be moved to white clover fields when desired in whatever numbers desired for pollination. This permits greater dependability than is permitted with wild bees. The best criterion for adequate pollination is either the appearance of the crop or the activity of the bees. Carmel, bees belonging to Hymenoptera comprised 90% of the pollinating fauna. The insect visitors are found greatly influenced by environmental factors like temperature, relative humidity, and light intensity. Honeybees are found to be mostly affected by temperature and humidity (13, 20).

During the development of the proposed research, within each objective, the following observations, determinations, and analyses were carried out:

Determination of phytomasse capacity according to the structure of experimental variants [19]: the production of phytomass is determined in green mass and dry matter (by production cycles and at each mowing) each growing year; from each experimental plot, green mass samples will be taken for various laboratory analyses.

The method of repeated mowing has as its principle the determination of the production of a small plot, significant for the parcel in question, each time the pasture is used. For this, plots of 2.5 to 10 m^2 are delimited, their number depending on the size of the parcel and its uniformity. In uniform pastures, the number of these plots is 4, and in uneven ones it is 10. If free grazing is practiced, the demarcated portions are much larger (100 m^2).

The total grass yield shall be obtained by adding together the yields obtained after each scythe during the growing season:

$$\mathbf{P_t = P_{c1} + P_{c2} + P_{c3} + \dots P_{cn}}$$

where:

P_t – total production;

$P_{c1.....cn}$ – production of the first scythe and so on.

However, the animals do not graze the entire quantity of grass available on the meadow, therefore after grazing, other areas are demarcated that are mowed, the grass is weighed and by summing up all the scythes to obtain the total quantity of uneaten plants:

$$\mathbf{P_n = P_{nc1} + P_{nc2} + P_{nc3} + \dots P_{ncn}}$$

where:

P_n – total unconsumed production;

$P_{nc1.....ncn}$ – unconsumed production of the first scythe and so on.

From these data it is possible to obtain the actual (PR) or actual production of the pasture, given by the relationship:

$PR = P_t - P_n$

The use coefficient (K) is then calculated according to the formula:

$$\mathbf{K} = \frac{\mathbf{PR}}{\mathbf{PT}(\%)}$$

This coefficient is different depending on the meadow.

If free grazing is practised on the grassland area under analysis, the actual production is based on the use coefficient according to the formula:

$$\mathbf{P_R} = \frac{\mathbf{P_T x K}}{100}$$

This is because it is difficult to quantify unconsumed production. The coefficient of use in this case is calculated according to the formula:

$$\mathbf{K} = \frac{\mathbf{M}}{\mathbf{N}}\mathbf{x}100$$

where:

M – weight of consumable plants N

– total weight of plants.

Pasture production, determined in green mass harvested in dry weather without dew, may be converted into dry matter or nutrient units on the basis of coefficients or by laboratory determinations (13). The choice of the method of determining the nutrient units in the feed obtained on the meadow is made according to the accuracy that is needed in the processing of data. By transformation with the help of coefficients, useful data are obtained in a short time, while laboratory determination involves additional time and cost. Thus, by indirect determination the ratio of the green mass on the pasture to the corresponding dry matter is generally 5:1. For the calculation of the dry matter divide the production of green mass by 5.

It attaches increasing importance to the mineral composition of the feed, which is in a direct relation with its net energy for milk (NEL) expressed in MJ, GJ, NEL (a notion derived from physics and defined by James Prescott Joule, English physicist (1818-1889). NEL is often calculated from the TDN value it is generated from the percentage of ADF. Usually in the case of increasing the percentage of ADF in the feed, the net energy for lactation decreases. There is currently a growing demand for healthier and safer food in order to protect the consumer. This involves improving the nutritional value and safety of food products, both those of plant and animal origin. Taking into account the obtaining of products of animal origin, it is very important the relationship that is established between the soil – plant – animal and implicitly the production obtained (13).

Conclusions

From the research carried out, it has been recorded that white clover is preferred for its proprieties for fixing atmospheric Co2, including decorative lawns. White clover maintains moisture and helps to retain carbon dioxide. Farmers who grow white clover among the grass in their fields can significantly reduce the carbon footprint of their dairy products. However, long-term studies are still needed to understand whether this phenomenon can be beneficial without affecting the other plants on mixed land by fixing CO2 in the soil. In order to exploit the potential of rhizobia for symbiotic fixation of atmospheric nitrogen, it is recommended to grow according to the pH of the soil, soil analysis being a mandatory step for setting up a crop.

Acknowledgments

This work appeared within the project: Program for increasing performance and innovation in doctoral and postdoctoral excellence research - PROINVENT, Contract no. 62487/03.06.2022 POCU/993/6/13 - SMIS code: 153299.

References

1. **Hărmănescu, M., Moisuc, A.,** The influence of organic and mineral fertilization on the sodium, potassium, calcium and magnesium content in the forages from a permanent meadow (Grădinari; Caraş-Severin), Research Journal of Agricultural Science, 2009, 41, 1.
2. **Hodgson, J.,** Grazing management. Science into practice, Longman Group UK Ltd, Harlow, Essex, UK, 1990.
3. **Holmes, W.,** Grass, its Production and Utilization, Oxford Blackwell Scientific Publications, UK, Oxford, 1989.
4. **Langlands, J.P., Holmes, C.R.,** The nutrition of ruminants grazing native and improved pastures. I, Seasonal variation in the diet selected by grazing sheep and cattle, Australian Journal of Agricultural Research, 1978, 29, 4.
5. **Leconte, D., Laissus, R.,** Studies of the growth of Trifolium repens, Fourrages, 1985, 103.
6. **Ledgard, S.F., Brier, G.J., Littler, R.A.,** Legume production and nitrogen fixation in hill pasture communities, New Zealand journal of Agricultural research, 1987, 30, 4.
7. **Moga, I., Schitea, M., Mateiaş, M.,** Plante furajere, Ed. Ceres, Bucureşti, 1996.
8. **Moisuc, A.I., Dukic, D.,** Cultura plantelor furajere, Ed. Orizonturi Universitare, Timisoara, România, 2002.
9. **Moisuc, A., Samfira, I., Cojocariu, L., Horablaga, M., Durău, C., Sărăţeanu, V., Laieş, D.G.,** Influence of fertilisation on the chemical composition of the biomass of grassland from grădinari (Caraş-Severin county), Research Journal of Agricultural Science, 2008, 40, 1.
10. **Norris, I.B.,** Floral initiation of white clover (Trifolium repens L.) in response to day length and temperature, Annals of Botany, 1987, 59, 2.
11. **O'Donovan, M., Dillon, P., Rath, M., Stakelum, G.,** A comparison of four methods of herbage mass estimation, Irish Journal of Agricultural and Food Research, 2002, 41, 17-27.
12. **Pate, J.S., Atkins, C.A., White, S.T., Rainbird, R.M., Woo, K.C.,** Nitrogen nutrition and xylem transport of nitrogen in ureide-producing grain legumes, Plant Physiology, 1980, 65, 5.
13. **Popescu, N., Popa, G., Stănescu, V.,** Determinări fizico-chimice de laborator pentru produsele alimentare de origine animală, Ed. Ceres, Bucureşti, 1986.

14. **Puia, I., Popescu, V., Resmerita, I., Erdelyi, S., Tarau, V., Tucra, I., Klemm, H.,** Cercetări privind înființarea pajiștilor cultivate în Transilvania, Lucrări științifice, Seria agricultura, Cluj-Napoca, 1972.
15. **Resmeriţă, I., Puia, I., Boşcaiu, N., Csürös, Ş.T.,** Monografia trifoiului alb din România, Ed. Academiei, Bucureşti, 1973.
16. **Resmeriţă, I., Pop, I.,** Consideraţii fitocenologice asupra pajiştilor de Trifoi, Institutul de Biologie Bucureşti, Academia Română, București, 1972.
17. **Samfira, I., Moisuc, A.,** Ecopratotehnica, Editura Eurobit, Timisoara, 2007.
18. **Samfira, I., Maruşca, T., Moisuc, A., Hărmănescu, M., Herbei, M., Sărăţeanu, V., Popescu, C.,** Elemente metodologice aplicate în cercetarea pajiştilor, Editura Mirton Timişoara, 2011.
19. **Şumălan, R.,** Fiziologie vegetală, Ed. Eurobit, Timișoara, 2006.
20. **Unkovich, M., Herridge, D., Peoples, M., Cadisch, G., Boddey, B., Giller, K., Chalk, P.,** Measuring plant-associated nitrogen fixation in agricultural systems, Ed. Australian Centre for International Agricultural Research, Australia, 2008.
21. **Warembourg, F.R., Lafont, F., Fernandez, M.P.,** Economy of Symbiotically Fixed Nitrogen in Red Clover (Trifolium pratenseL.), Annals of Botany, 1977, 80, 4.

SOME ANATOMOPATHOLOGICAL LESIONS OF *PASTEURELLA* INFECTIONS IN RABBITS

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Summary

The paper presents the results of the necropsy examinations that were made on a total of 37 dead rabbits, with pasteurellosis as the cause of death, from 7 locations, females and males, of different ages. Most of the rabbits presented specific signs of pasteurellosis before death, symptoms such as sneezing, ocular secretions, and whitish nasal. Some of the rabbits presented subcutaneous abscesses, the most frequent locations of abscesses were in the head, neck, and limbs, both in the thoracic and pelvic limbs. Abscesses on the nipple chain, otitis, torticollis, and dental infection were observed, but with a lower frequency. At the necropsy examination characteristic lesions of pasteurellosis were highlighted, lesions such as active pulmonary congestion and fibrinous pleurisy, pyometra, abscesses on the lungs, hydrothorax, pyothorax, peritonitis, and abscesses. The histopathological examination revealed massive interstitial leukocyte infiltrations and the presence of abundant fibrin deposits at the tissue level. The diagnosis was confirmed by a bacteriological examination.

Keywords: *Pasteurella*, rabbits, lesions, abscesses, histopathological.

Pasteurellosis is a common respiratory disease and a major cause of morbidity in rabbits. It is also known as hemorrhagic septicemia of rabbits in the specialized literature, and it is commonly encountered in intensive and traditional breeding, with most adult rabbits considered carriers (2, 9, 10).

Pasteurella multocida is the primary etiologic agent implicated; the bacteria are most commonly associated with respiratory infections, but they can also cause subcutaneous infections, infections in various organs, and even septicemia (1, 4, 5, 6).

Rabbit respiratory infections are a serious problem for rabbit breeders due to high mortality, sudden death, increased treatment costs, and weight loss (6, 15).

The disease typically occurs spontaneously, through self-infection with commensal germs, in rabbit specimens exposed to favorable factors such as cold, air currents, dampness, the presence of harmful gases, after transport, and abrupt changes in nutrition (2, 6).

The lesions discovered during a necropsy differ depending on the disease's form and the location of the infection (3, 17). The most common manifestations of this disease are coryza and pasteurelic nasal catarrh (1, 6, 11, 16).

Materials and methods

The research was conducted on seven traditional rabbit breeders in Romania's western region. Three of these breeders are members of rabbit breeders who frequently exhibit at professional exhibitions.

There were 277 females and males of the following breeds in the herds studied: *German Giant Pied, German Giant, Rex, Pannonian White, German Ram, Small Pied,* and *Dwarf Pied*, aged 2 months to 3 years (Table 1).

Clinical signs of pasteurellosis were observed in the rabbit population belonging to these breeders, and some rabbits even died.

The dead specimens were collected in order to be examined via necropsy (Table 2).

Table 1

Distribution of rabbits by location, and number of deaths

	A		B		C		D		E		F		G		Total	
Total number of rabbits	63		77		52		29		35		14		7		277	
Gender M/F	14	49	27	50	14	38	6	23	9	26	3	11	2	5	75	202
Pneunomia	2	5	2	5	0	1	0	0	1	1	0	0	0	0	5	12
Rhinitis	1	3	1	4	2	3	0	0	1	2	0	0	0	1	5	13
Sinusitis	1	0	1	2	1	1	0	0	1	0	0	0	0	0	4	3
Metritis	0	4	0	2	0	0	0	1	0	1	0	0	0	0	0	8
Otitis	1	1	2	0	0	0	0	0	0	1	0	0	0	0	3	2
Subcutaneous abscess	0	5	2	6	1	4	0	1	0	2	0	0	0	1	3	19
Pulmonary Congestion	2	1	0	0	1	0	0	0	0	0	0	0	0	0	3	1
Dental Infections	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Pyometra	0	3	0	2	0	2	0	0	0	0	0	0	0	1	0	8
Pyothorax/Hydrothorax	2	1	0	0	0	2	1	0	0	0	0	0	0	0	3	3
Peritonitis	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	2

To determine the etiological agent, epidemiological studies, clinical examinations, necropsies, and laboratory tests were carried out.

Table 2

Distribution of lesions in the locations

Locations	A		B		C		D		E		F		G		Total	
Total number of rabbits	63		77		52		29		35		14		7		277	
Gender M/F	14	49	27	50	14	38	6	23	9	26	3	11	2	5	75	202
Mortality	2	5	7	6	2	6	0	2	2	4	0	0	0	1	13	24
Total Mortality	7		13		8		2		6		0		1		37	

Results and discussions

The majority of the animals were cachectic, and some had subcutaneous abscesses, according to a general examination of the corpses.

In the head and neck region, in addition to subcutaneous abscesses, dental infections were also revealed, leading to the formation of a ventral fistula of the eyes, the eyeball being compromised, and otitis (Fig.1).

Fig. 1. Dental abscess secondary to infection with *Pasteurella multocida*

There were lesions in the thoracic cavity such as hydrothorax, a cloudy liquid with caseous deposits, and pyothorax (Fig. 2); in most cases, the thoracic cavity walls were infected. Abscesses ranging in size from a grain of corn to the size of walnut were discovered in pulmons in some specimens.

The contents of the abscesses were whitish and paste-like in consistency. There have been reports of abscesses that were larger than the pulmon and that engulfed the entire lung. In addition to abscesses, pulmonary congestion, fibrinous pleuritis, pulmonary emphysema, and fibrinous bronchopneumonia were discovered in the lungs (Fig. 3, Fig. 4).

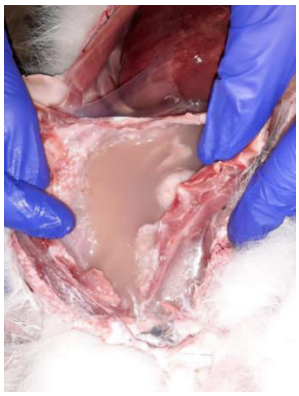

Fig. 2. Pyothorax with the presence of a considerable amount of fluid in the chest cavity

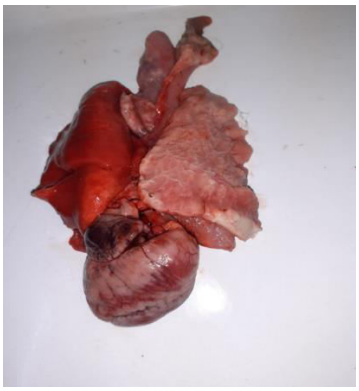

Fig. 3. Active pulmonary congestion and fibrinous pleurisy

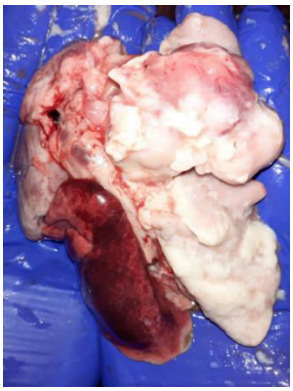

Fig. 4. Passive pulmonary congestion and multiple lung abscesses

Some corpses had peritonitis and ascites in the abdominal cavity. Furthermore, caseous deposits on the organs in the abdominal cavity formed adhesions between the organs in some cases (Fig. 5, Fig. 6).

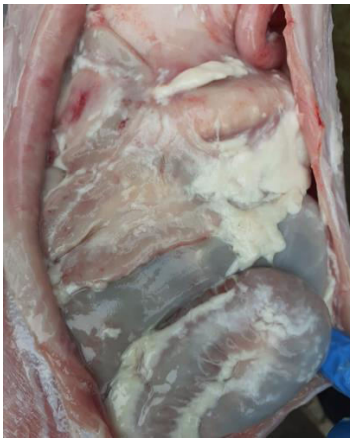

Fig. 5. Peritonitis with fibrin deposits and adhesions between organs

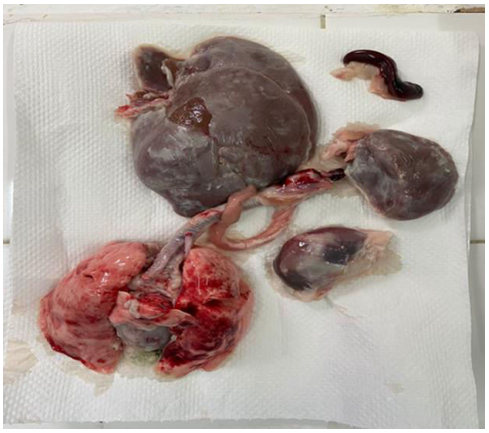

Fig. 6. Liver, kidney, and spleen enlarged, liver and kidney showing fibrin deposits on the surface, lungs with fibrinous bronchopneumonia

The females had lesions in their reproductive systems. Metritis and pyometra were two different types of injuries.

Some specimens' uterine bodies and horns grew in volume, color, shape, and consistency, and an abundant, caseous, whitish deposit of paste consistency was discovered at the opening of these anatomical formations. In some cases, the uterine horns fistulized and the contents poured into the abdominal cavity (Fig. 7, Fig. 8).

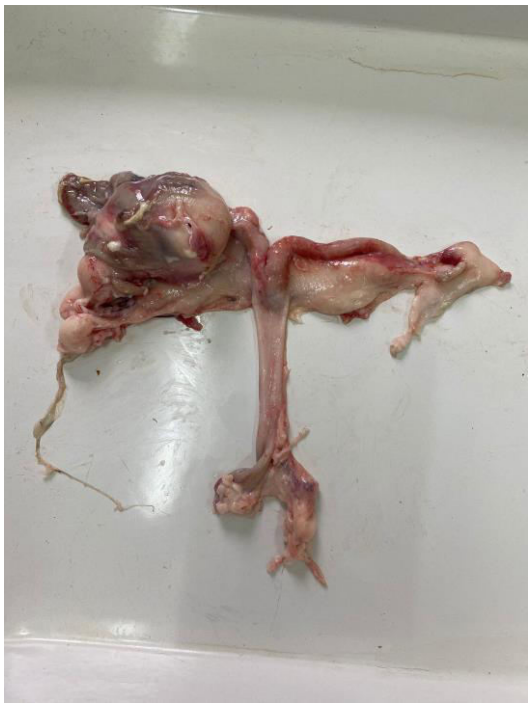

Fig. 7. Pyometra on the left uterine horn with areas of necrosis and the presence of a fistula

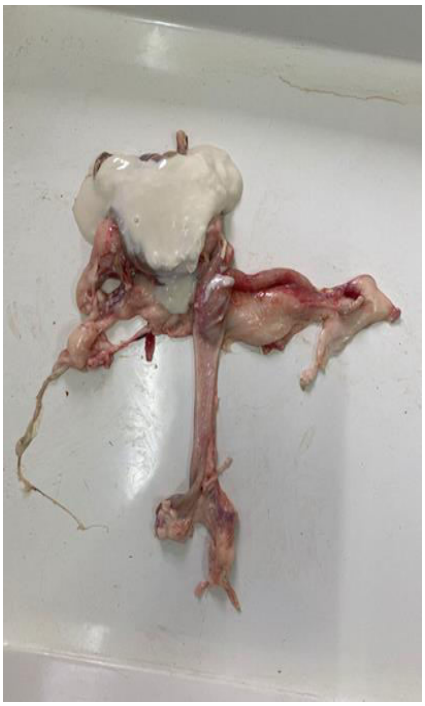

Fig. 8. The section view of the uterine horn and the presence of an abundant deposit of gray-white caseous exudate

A one-month-old rabbit from a batch of rabbits imported from Taiwan was studied in Japan in 2019 (7).

Characteristic lesions such as suppurative pleuropneumonia, fibrin deposits over the lungs, color modification in the lungs, and multiple coagulative necroses were discovered. The histopathological examination revealed necrosis of the alveolar walls and coagulative necrosis with bacterial cells, macrophages, and neutrophils surrounding the necrotic area. Other studies have reported lesions of

fibrinonecrotic pneumonia, fibrinous pleurisy, fibrinous pericarditis, and endocarditis. Metritis, often accompanied by vaginitis, salpingitis, and peritonitis, as well as pyometra, was observed in females at the reproductive system level. When the uterus was opened, an abundance of purulent yellowish-white content was discovered. Mastitis can also occur, which promotes the development of abscesses in the mammary gland and abscesses (1, 5, 10, 12, 13, 14, 18, 19, 20).

Otitis, encephalitis, and meningoencephalitis were also discovered, as well as dacryocystitis, and by extending the infectious process, the dental roots and teeth in question were also affected in some cases (1, 4, 5, 8, 14, 16, 17).

Conclusions

The necropsy revealed a number of lesions in a variety of locations affecting the respiratory, digestive, and reproductive systems. Infections with bacteria of the genus *Pasteurella* frequently result in life-threatening injuries.

Hydrothorax, pyothorax, passive or active pulmonary congestion, lung abscesses, subcutaneous abscesses, otitis, peritonitis, metritis, and pyometra are the primary lesions highlighted.

Bacteriological testing confirmed the diagnosis.

References

1. **Coudert, P., Rideaud, P., Virag, G., Cerrone, A.,** Pasteurellosis in Rabbits, Melle, Ilvo, 2006.
2. **Dărăbuş, G., Cătană, N.,** Bolile infecţioase și parazitare ale iepurilor, Ed. Brumar, Timișoara 1996.
3. **Fodor, I., Coman, M., Catana, N.,** An Outbreak of Marek's Disease in Broiler Chickens: Epidemiological, Clinical and Anatomopathological Aspects, Lucrări Ştiinţifice Medicină Veterinară Timişoara, 2009, XLII, 1, 224-227.
4. **Herman, V., Moga Mânzat, R., Rămneanţu, M.,** Diagnosticul în bolile infecţioase ale animalelor, Ed Mirton Timisoara, 2006.
5. **Iancu, I., Pascu, C., Degi, J., Cătana, N., Stancu, A., Martin, A., Herman, V.,** caracterizarea unor tulpini de pasteurella şi mannheimia isolate din pulmoni de viţel cu afecţiuni respiratorii, Lucrări Ştiinţifice Medicină Veterinară Timisoara, 2019, 54, 528-532.
6. **Iorgoni, V., Iancu, I, Pascu, C., Costinar, L., Degi, J., Hulea, A., Gligor, A., Nicula, M., Herman, V.,** Research in pasteurellosis of domestic rabbits from extensive growing, Lucrări Ştiinţifice Medicină Veterinară Timisoara, 2022, LV, 1, 105-113.
7. **Kawamoto, E., Sawada, T., Maruyama, T.,** prevalence and characterization of pasteurella multocida in rabbits and their environment in Japan, Nihon Juigaku Zasshi, 1990, 10, 52, 5, 915-21.

8. **Mohamed, F.M., Mansy, M.F., Hassan, A.K.,** Antibacterial Sensitivity and detection of virulence associated gene of *Pasteurella multocida* isolated from rabbits, Journal of World's Poultry Research, 2020, 10, 2, 165-171.
9. **Nakagawa, M., Nakayama, K., Saito, M., Takayama, S., Watarai, S.,** Bacteriological and serological studies on *Pasteurella multocida* infection in rabbits, Jikken Dobutsu, 1986, 10, 35, 4, 463-469.
10. **Palócz, O., Gál, J., Clayton, P., Dinya, Z., Somogyi, Z., Juhász, C., Csikó, G.,** Alternative treatment of serious and mild *Pasteurella Multocida* infection in New Zealand white rabbits, BMC Veterinary Research, 2014, 10, 276.
11. **Pascu, C., Herman, V., Iancu, I., Costinar, L.,** Etiology of mastitis and antimicrobial resistance in dairy cattle farms in the western part of Romania, Antibiotics, 2022, 11, 1, 57-69.
12. **Paul, I.,** Etiomorfopatologia Bacteriozelor la Animale, Ed. Pim, Iași, 2005.
13. **Premalatha, N., Kumar, K.S., Purushothaman, V., Ravikumar, G., Murali Manohar, B.,** Incidence of pasteurellosis snuffles in a rabbit farm. Tamilnadu, Journal of Veterinary and Animal Sciences, 2009, 5, 269-271.
14. **Stahel, A.B., Hoop, R.K., Kuhnert, P., Korczak, B.M.,** Phenotypic and genetic characterization of *Pasteurella multocida* and related isolates from rabbits in Switzerland, Journal of Veterinary Diagnostic Investigation, 2009, 21, 793-802.
15. **Takashima, H., Sakai, H., Yanai, T., Masegi, T.,** Detection of antibodies against *Pasteurella multocida* using immunohistochemical staining in an outbreak of rabbit pasteurellosis, Journal of Veterinary Medical Science, 2001, 63, 171-174.
16. **Uenoyama, K, Ueno, Y, Tosaki, K.,** Immunohistochemical and molecular analysis of *pasteurella multocida* in a rabbit with suppurative pleuropneumonia, Journal of Veterinary Medical Science, 2020, 82, 1, 89-93.
17. **Varga, M.,** Infectious disease of domestic rabbits, Textbook of Rabbit Medicine, Butterworth-Heinemann, 2014.
18. **Vigneshwar, R., Imayarasi, K., Divisha, R.,** A rare case report of *Pasteurella multocida* infective valvular endocarditis in a laboratory rabbit, Pharma Innovation Journal, 2020, 9, 12, 82-85.
19. **Wang, J., Sang, L., Sun, S.,** Characterization of *pasteurella multocida* isolated from dead rabbits with respiratory disease in Fujian, China, BMC Veterinary Research, 2019, 15, 438.
20. **Wilkie, I.W., Harper, M., Boyce, J.D., Adler, B.,** *Pasteurella multocida*: diseases and pathogenesis. Current Topics in Microbiology and Immunology, 2012, 361, 1-22.
21. **Yang, W., Li, M., Zhang, C., Zhang, X., Guo, M., Wu, Y.,** Pathogenicity, colonization, and innate immune response to *Pasteurella multocida* in rabbits, BMC Veterinary Research, 2022, 18, 1, 416.

STUDY REGARDING THE IMPORTANCE OF COLOSTRAL FEEDING IN NEW BORN CALVES

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Summary

At the moment of parturition, the calf makes contact for the first time with the new environment, being subjected to a continuous adaptation of the organism. Due to the fact that it does not have a well-developed immune system, in order to defend the body against infectious agents, it is very sensitive to diseases and prone to stressful factors, including the possibilities of proper feeding. Therefore, the main issues that must be considered are the administration of quality colostrum and the optimal passive transfer of immunity from mother to newborn. Colostrum is the first food to which the newborn has access, being considered indispensable for its optimal growth and development. Calves are born with a deficiency in vitamin A at the hepatic level and a major immune deficiency, because at the placental level, during intrauterine development, immunoglobulins and essential vitamins cannot cross the barrier. The properties of colostrum are due to its rich content in IgG_1 and IgM immunoglobulins. In addition to the role of immune protection, colostrum also contains a series of non-specific antimicrobial factors: lysozyme, lactoferrin, lactoperoxidase/ thiocyanate/ hydrogen/ peroxide, xanthine oxidase, vitamin A, B12 and folic acid. Another important characteristic is the absorption capacity at the intestinal level compared to the immunized components and its dynamics. Thus, intestinal permeability is limited in time, being maximum in the first 6 hours of life, then gradually decreasing. Failure to comply with this short period of time can cause hypoimmunoglobulinemia, severe disease and high percentage of cattle mortality.

Keywords: cattle, colostrum, feeding.

Colostrum is the first form of breast milk released after parturition, being rich in micro- and macronutrients, immunoglobulins, antimicrobial peptides and growth factors. It also has a very important role in optimal intestinal development and functioning, more specifically in microbiome regulation and neonatal intestinal immunological maturation.

Colostral feeding management has a significant impact on optimal growth and development because postpartum calves go through a critical period that can affect their health and welfare.

Materials and methods

In the present research, 20 bibliographic sources were consulted, which had as a subject of study the influence of colostrum on the bovine calf.

High morbidity and mortality among young neonatal cattle is still a major problem at world herd level, being excessively high in early calf growth (7).

Neonatal mortality can vary worldwide between 8% and 64%, its incidence being defined as the duration from the moment of birth to 48 hours postpartum. Several recent studies have revealed that in Germany, mortality rates on dairy farms can reach up to 17% loss of calves up to 6 months of age, and in the USA morbidity rates can reach up to 34% and mortality up to 5% of cattle herds before weaning (8, 11).

Severe diarrhea remain the main factor in the mortality of pre-weaned cattle, among cattle herds, but the digestive syndrome can present several causes such as low growth rate or decreased milk production in primiparous cows, consequently generating major economic losses. It is a multifactorial disease resulting from cumulative exposure to environmental infectious agents and host immunity. The most frequent diarrheal etiological agents encountered are represented by: rotavirus, coronavirus, *Cryptosporidium parvum*, enterotoxigenic *Escherichia coli*, *Clostridium perfringens* and *Salmonella spp*. Exposure to these factors can cause an exaggerated diarrhea, on a weakened immunological background as a result of other diseases or from due to poor management (9, 12, 13, 15).

The usual therapy used in the case of neonatal diarrhea can be represented by broad-spectrum antimicrobials, but it is necessary to develop alternative treatments to avoid the antimicrobial resistance generated by the frequent use of anti-infective medication and thus avoid the negative impact on the intestinal microflora of the calf (2, 6).

Bovine colostrum is abundant in immune and bioactive factors that improve immune function and development. This abundant and natural combination of immunoglobulins, growth factors, natural antimicrobial factors and nutrients may represent an optimal alternative to replace the usual broad-spectrum antimicrobial therapy in the case of diarrhea in dairy cattle (3, 4).

In addition to the immunological component with an effect on the passive immunity of newborn calves, colostrum also contains a high number of immunomodulatory peptides that can affect the neonatal immune response. Some of these factors are provided by colostral immune cells that are involved in establishing local and systemic neonatal immunity. In addition, the potential effects of colostrum on the neonatal gut microbiome are likely and becoming more important in calf nutrition research (5, 10, 13).

Colostrum works to help promote the growth and development of the neonatal calf through immune function in the first hours of life, so it is vital to monitor the quality, quantity and chastity of colostrum and ensure that the calf receives its first feeding of colostrum in a timely manner. To be characterized as “good quality”, colostrum must contain >50 g/L immunoglobulin G (IgG) to ensure successful transfer of passive immunity. Quality can be altered by many factors, such as breed and age of the female, calving season, duration of previous lactation and delayed colostrum collection. In addition, nutrition and health during the periparturient period of the mother, such as energy and duration of the dry period, have been shown to affect IgG levels in colostrum. In terms of quantity, calves should receive 10–12% of body weight of colostrum at first feeding and ensure consumption of at least 150–200 g of

IgG during the first 24 h of life. Due to the intestinal closure that occurs on the first day of life, it is vital that colostrum is offered immediately after birth to provide the calf with the immunoglobulins necessary for immune support (10, 12, 14, 17).

The method by which colostrum is collected and stored can influence the metabolism, endocrine system and nutrition of cattle. Most dairy farmers reported that they regularly remove calves from the farrowing pen within 30 minutes of birth and prohibit nursing from the mother. If contamination occurs during the collection process, bacteria can bind to immunoglobulins, inhibiting their transport through enterocytes and thus reducing the transfer of passive immunity (5, 8, 18).

The maturation and function of the neonatal gut allows the calf to digest and absorb the nutrients provided by the colostrum. Therefore, colostrum intake supports the triggering of anabolic processes in several tissues, stimulating postnatal body growth and organ development. After the colostrum feeding period, an intensive milk feeding protocol, i.e. at least 20% of the milk intake per day, is required to achieve the calf's growth spurt and organ development during the pre-weaning period. Insufficient intake delays postnatal growth and can have deleterious effects on the development of organs, for example, the intestine and the mammary gland. The somatotropic axis as the main postnatal endocrine regulatory system for body growth is stimulated by the intake of large amounts of colostrum and milk and indicates the promotion of anabolic metabolism in calves. The development of the pre-gastric compartments is an important issue during the pre-weaning period in calves, and their maturation is achieved through solid feed intake. Unfortunately, intensive milk feeding programs compromise solid food intake in the first weeks of life (10, 13, 19).

In order to achieve an intensive milk feeding program, it is recommended that the weaning process does not start early and that the intake of solid food is largely adapted to the needs of each individual. A feeding concept based on intensive milk feeding prevents the feeling of hunger and abnormal behavior of the calves. Milk performance studies in dairy cows indicate that feeding management during early calf growth influences lifetime performance. Therefore, an intensive milk feeding program affects both immediate and long-term performance (1, 8).

Bovine colostrum is much richer in immunoglobulins, with a high content, approximately more than 100 times higher than in mature milk, being essential for protection against various diseases to which the newborn calf is exposed. Common molecules may include immunoglobulin M (IgM), immunoglobulin A (IgA) and IgG, of which IgG is the primary isotope accounting for 85–90% of all bovine colostral immunoglobulins (12, 13, 14).

During the first 24 h of life, maternal immunoglobulins are absorbed from the colostrum to provide passive immunity to the calf. After ingestion, the immunoglobulins are transferred into the circulatory system of the newborn through the lumen of the small intestine, thus the calf receives immediate, short-term immunity. Because intestinal permeability decreases rapidly in the first day of life, delaying colostrum intake until 12 hours of life will dramatically reduce passive transfer of immunoglobulins. Likewise, immunoglobulins perform their role even before being absorbed and

transferred into the bloodstream, thus protecting the intestinal mucosa from various pathogenic agents, which can graft and intrude at the level of the intestinal mucosa membrane. Colostrum also contains compounds that are responsible for the protection of immunoglobulins, such as trypsin inhibitor, which is found in a very high concentration, which prevents the degradation of IgG and increases the availability of this molecule for the body (7, 14, 20).

In addition to providing immunoglobulins in colostrum, the latter contains an abundance of antimicrobial components, thus lactoferrin which is a bioactive protein in colostrum, prevents sepsis in infants and calves. Lactoferrin exhibits antimicrobial properties by creating an iron deficiency at the bacterial level, thus minimizing the potential for bacterial growth. Furthermore, it inhibits the growth of many infectious agents, including Escherichia coli and Salmonella spp. Lactoperoxidase is a similar bioactive compound that exhibits antimicrobial qualities by inhibiting bacterial metabolism by suppressing oxidation in protein groups. Another bioactive compound, lysozyme, can actively protect the host against Gram-positive and Gram-negative strains of bacteria by hydrolyzing β 1-4 bonds in the cell wall, thereby causing cell lysis (4, 8, 15, 20).

Colostrum is also rich in additional bioactive molecules such as insulin, insulin-like growth factor-I (IGF-I), and IGF-II. After birth, their concentrations drop drastically. Therefore, early collection of colostrum is vital to provide the body with concentrations of both IgG and bioactive molecules. Insulin has been shown to improve the calf's oral glucose absorption capabilities, thereby increasing electrolyte levels. Growth factors also stimulate the development and maturation of the intestines. Specifically, the growth and development of epithelial cells is stimulated by IGF-I and IGF-II. Similarly, colostrum is rich in additional bioactive factors related to the innate and acquired immune system that are essential in preventing and treating the body against certain diseases. These include neutrophils, macrophages, immune regulators and anti-inflammatory molecules. Neutrophils and macrophages are responsible for the elimination of pathogens, directly through phagocytosis, as well as through the production of cytokines (including IL-1β, IL-6, TNF-α and INF-γ). In addition to factors that provide direct antimicrobial support with endotoxin-neutralizing abilities, colostrum contains several bioactive molecules, such as leptin, casein, and α-lactalbumin, which can reduce intestinal inflammation to encourage tissue repair and improve integrity of gastrointestinal mucosa (1, 14, 16, 17).

Results and discussions

Colostrum is a much more concentrated source than milk that contains 2 times more nutrients, 5 times more protein, 1.7 times more fat and higher amounts of minerals and vitamins, especially vitamin A. The large amount of constituent proteins is due to the presence of immunoglobulins, especially IgG, which represents the indispensable component with a vital role in the body's defense.

The concentration of colostrum in antibodies varies between 2 and 23%, but an important factor is the mother's exposure to infectious agents during the last month of pregnancy.

An important characteristic is the bacteriostatic effect with a role in non-specific defense against infections, thus antimicrobial factors play a primary role against Gram-positive and Gram-negative bacteria, aerobic and anaerobic, fungi and viruses. It also contains a series of carbohydrates for energy support (glucose, fructose, glucosamine), proteins, growth factors, enzymes, vitamins, especially vitamin A and E.

Conclusions

Colostrum is the first food to which the newborn has access, being considered indispensable for its optimal growth and development

Neonatal and pre-weaning pathology can still represent a major problem worldwide, in breeding herds. An increased supply of colostrum, immediately after birth, ensures optimal growth and development of the calves and protects the body to avoid morbidity, even neonatal morality.

Colostrum has great potential regarding its use as a natural prophylactic and as an innovative therapy for the various digestive ailments that can occur postpartum.

Welfare and growth management can be improved by developing modulated therapy with antimicrobials and colostrum, in order to prevent antibiotic resistance at the farm level.

An intensive milk feeding program starting immediately after birth with increased colostrum intake and subsequent intensive milk feeding supports the postnatal growth and development of calves, prevents behavioral abnormalities and promotes optimal growth of young animals.

References

1. **Barry, J., Bokkers, E.A.M., Berry, D.P., de Boer, I.J.M., McClure, J., Kennedy, E.,** Associations between colostrum management, passive immunity, calf-related hygiene practices, and rates of mortality in preweaning dairy calves, Journal of Dairy Science, 2019, 102, 10266-10276.
2. **Carter, H.S.M., Renaud, D.L., Steele, M.A., Fischer-Tlustos, A.J., Costa, J.H.C.,** A Narrative Review on the Unexplored Potential of Colostrum as a Preventative Treatment and Therapy for Diarrhea in Neonatal Dairy Calves, Animals, 2021, 11, 8, 2221.
3. **Codreanu, I.** Animal physiology, Ed. Printech, Bucuresti, 2020.
4. **Constantin, V., Gheorghe, M.,** Tratat de cresterea bovinelor, Risoprint, Cluj-Napoca, 2012.

5. **Eckert, E., Brown, H.E., Leslie, K.E., DeVries, T.J., Steele, M.A.,** Weaning age affects growth, feed intake, gastrointestinal development, and behavior in Holstein calves fed an elevated plane of nutrition during the preweaning stage, Journal of Dairy Science, 2015, 98, 9, 6315-6326.
6. **Gelsinger, S.L., Heinrichs, A.J., Jones, C.M.,** A meta-analysis of the effects of preweaned calf nutrition and growth on first-lactation performance, Journal of Dairy Science, 2016, 99, 8, 6206-6214.
7. **Godden, S.M., Lombard, J.E., Woolums, A.R.,** Colostrum Management for Dairy Calves, Food Animal Practice, 2019, 35, 3, 535-556.
8. **Hammon, H.M., Liermann, W., Frieten D., Koch, C.,** Review: Importance of colostrum supply and milk feeding intensity on gastrointestinal and systemic development in calves, Animals, 2020, 14, 1, s133-s143.
9. **Hao, Y., Guo, C., Gong, Y., Sun, X., Wang, W., Wang, Y., Yang, H., Cao, Z., Li, S.,** Rumen Fermentation, Digestive Enzyme Activity, and Bacteria Composition between Pre-Weaning and Post-Weaning Dairy Calves, Animals, 2021, 11, 9, 2527.
10. **Hue, D.T., Skirving, R., Chen, T., Williams, J.L., Bottema, C.D.K., Petrovski, K.,** Colostrum source and passive immunity transfer in dairy bull calves, Journal of Dairy Science, 2021, 104, 7, 8164-8176.
11. **Lindsey, E., Hulbert, S., Moisá, J.,** Stress, immunity, and the management of calves, Journal of Dairy Science, 2016, 99, 4.
12. **Lopez, A.J., Heinrichs, A.J.,** Invited review: The importance of colostrum in the newborn dairy calf, Journal of Dairy Science, 2022, 105, 4, 2733-2749.
13. **Menchetti, L., Traina, G., Tomasello, G., Casagrande-Proietti, P., Leonardi, L., Barbato, O., Brecchia, G.,** Potential benefits of colostrum in gastrointestinal diseases, Frontiers in Bioscience, Scholar, 2016, 8, 331-351.
14. **Playford, R.J., Weiser, M.J.,** Bovine Colostrum: Its Constituents and Uses, Nutrients, 2021, 13, 265.
15. **Raboisson, D., Delor, F., Cahuzac, E., Gendre, C., Sans, P., Allaire, G.,** Perinatal, neonatal, and rearing period mortality of dairy calves and replacement heifers in France, Journal of Dairy Science, 2013, 96, 5, 2913-2924.
16. **Rathe, M., Müller, K., Sangild, P.T., Husby, S.,** Clinical applications of bovine colostrum therapy: a systematic review, Nutrition Reviews, 2014, 72, 4, 237-254.
17. **Robbers, L., Jorritsma, R., Nielen, M., Koets, A.,** A Scoping Review of On-Farm Colostrum Management Practices for Optimal Transfer of Immunity in Dairy Calves, Frontiers in Veterinary Science, 2021, 8, 668639.
18. **Sangild, P.T., Vonderohe, C., Melendez, Hebib, V., Burrin, D.G.,** Potential Benefits of Bovine Colostrum in Pediatric Nutrition and Health, Nutrients, 2021, 13, 8, 2551.

19. **Stott, G.H., Marx, D.B., Menefee, B.E., Nightengale, G.T.,** Colostral immunoglobulin transfer in calves II. The rate of absorption, Journal of Dairy Science, 1979, 62, 11, 1766-1773.
20. **Uyama, T., Kelton, D.F., Winder, C.B., Dunn, J., Goetz, H.M.,** Colostrum management practices that improve the transfer of passive immunity in neonatal dairy calves: A scoping review, Plos One, 2022, 17, 6, e0269824.

EPIDEMIOLOGICAL RESEARCH REGARDING THE EVOLUTION OF TRICHINELLOSIS IN MARAMURES COUNTY (ROMANIA)

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Summary

Trichinellosis is a non-contagious, cosmopolitan parasitosis, found in several species of mammals and in humans, produced by nematodes that are part of the *Trichinellidae* family. Human trichinellosis in Romania is a zoonosis with a high incidence, with the consumption of uncontrolled pork and game being incriminated as the leading cause of human infection. The aim of the present study was to evaluate the evolution of *Trichinella* spp. infestation in wild boars from Maramureș County during 2017-2021. The study was carried out through the collaboration between the Faculty of Veterinary Medicine Timisoara and the Sanitary Veterinary and Food Safety Directorate from Baia Mare. The epidemiological data collected in the period 2017-2021 have been processed. The muscle samples from the boar have been examined by direct trichinelloscopy and artificial digestion. In 2020 highest number of positive cases was recorded from the total of muscle samples examined (7.29%), compared to the year 2021 in which the prevalence of trichinellosis in wild boar was 0. Concluding, the surveillance and control of *Trichinella* infection in wild boars must be maintained and promoted.

The important features of trichinellosis have been described in various bibliographic reports: identification of the sources and methods of infestation, establishing the links between the domestic and the wild cycle, the molecular description of species and genotypes, the correlation of clinical manifestations in humans with the results of epidemiological investigations, the demonstration of transplacental transmission of *Trichinella* larvae (1, 2, 6, 10, 21, 25).

Several species are involved in the production of the disease: *Trichinella* spiralis, *T. nativa, T. britovi, T. murrelli, T. nelsoni, T. pseudospiralis, T. papuae* (23, 24, 25). *Trichinella spiralis* is the most known and studied species with a great prevalence in the domestic pig, followed by other mammals: dogs, cats, foxes, wolves, wild boars, bears, mice, and rats, including humans (13, 23, 24).

The source of infestation for animals and humans is represented by animals that contain *Trichinella* larvae or cysts in their muscles (9, 11).

Epidemiological relationships in trichinellosis can be embodied in a domestic cycle in which the main actors are the pig and the rat; they are joined by the horse, the dog, and the cat. The second cycle is the sylvatic one and is represented by the boar, fox, and bear. The rat is the most important link between the cycles described previously. The domestic cycle has relevant importance for public health (2, 10).

In Romania, numerous studies have been reported an increased prevalence of *Trichinella* infection in wild species (5, 12, 15, 16). Of these, the wild boar is a significant source of human contamination. A local and regional study focused on this game species reported a prevalence varying between 0.1 and 23.5% (18).

In the period 2015-2019, in Maramureș, *Trichinella britovi* larvae were identified in badgers (*Meles meles*) (4). Also, in the period 2014-2015, larvae of *Trichinella spp*. were identified in the wild cat through artificial digestion (14).

In this descriptive table, the aim of the present study was to evaluate the evolution of *Trichinella* spp. infestation in wild boars from Maramureș County during 2017-2021.

Materials and methods

The study was carried out through the collaboration between the Faculty of Veterinary Medicine Timisoara and the Sanitary Veterinary and Food Safety Directorate from Baia Mare. The epidemiological data collected in the period 2017-2021 have been processed. The muscle samples from the boar have been examined by direct trichinelloscopy and artificial digestion.

Direct trichinelloscopy: from each of the diaphragm muscles, intercostal or abdominal muscles, two samples are collected, in total four samples of 20 grams for each carcass; one compressor of 28 sections is executed from each sample, a total of four compressors for each case; the sections are clarified, with 10% acetic acid or 9° food vinegar in the boar, and with sodium hydroxide 3%, in the bear. When the cysts are calcified, in both species, clarification is done with acetic acid 10% (26).

Artificial digestion method: the actual digestion of the muscle tissue and the release of the larvae: the sample is immersed in the digestion liquid, and the meat sample is homogenized continuously under stirring (with the magnetic stirrer) at a temperature of 44 - 46° C, for 30 - 60 minutes, until the muscle fragments disappear. To isolate the larvae, the digestion liquid is filtered, then rested for 30 minutes. The filtered liquid is passed through a 40 ml graduated test tube, which was left to rest for 10 minutes. Keep 10 ml of the filtrate to be examined (8).

Results and discussions

The unpublished data provided by the Sanitary Veterinary and Food Safety Directorate from Baia Mare revealed the following epidemiological situation highlighted in Table 1.

The year 2020 is the year in which the highest number of positive cases was recorded from the total of muscle samples examined: 14/192 (7.29%), compared to the year 2021 in which the prevalence of trichinellosis in wild boar was 0 (Fig. 2-3).

Table 1

Status of wild boar samples, 2017-2021

No. crt.	The year	Examination of samples by artificial digestion	Positive samples	Percent
1.	2017	344	24	6.98%
2.	2018	616	28	4.54%
3.	2019	474	19	4%
4.	2020	192	14	7.29%
5.	2021	152	0	0%
Total		1.778	85	22.81%

In 2017, the prevalence of trichinellosis in wild boars was 6.98%, followed by a decrease in 2018 (4.54%), respectively 2019 (4%), and in the following year, 2020, the prevalence of trichinellosis in wild boars was again increased (7.29%) (Fig. 1).

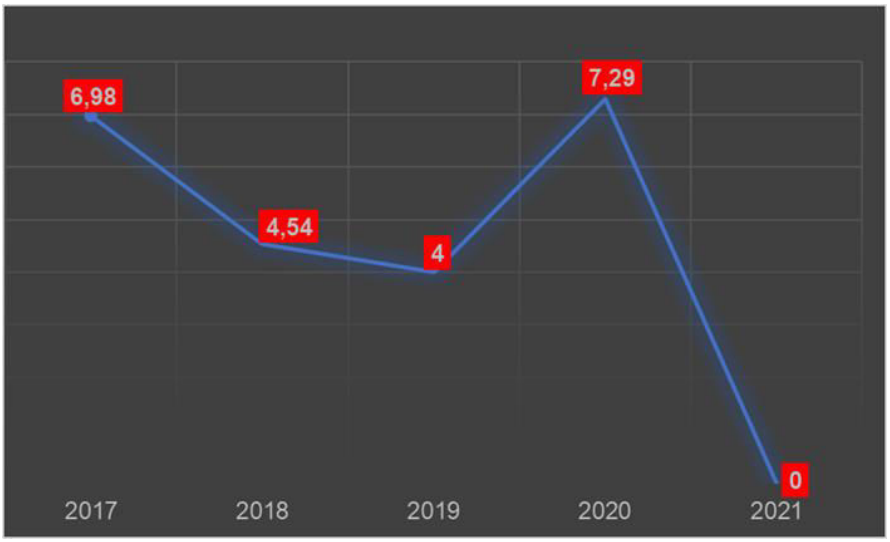

Fig. 1. The trichinellosis evolution in wild boars, 2017-2021

A number of 1778 muscle samples have been examined in the period 2017-2021, in Maramures County (Romania). The prevalence of trichinellosis in wild boars

was 22.81% (85/1.778).

Fig. 2. Cysts of *Trichinella* spp. by trichinelloscopic examination

Fig. 3. *Trichinella spp.* larvae by artificial digestion

Trichinellosis remains an important foodborne parasitic zoonosis with an annual incidence of approximately 10.000 clinical cases worldwide. It is one of the most serious zoonotic diseases in Romania, with over 28.000 human cases reported in the last 25 years. In this context, Romania remains the country with the highest degree of infestation with *Trichinella* worldwide (7).

T. spiralis is the predominant species in domestic animals, while *T. britovi* is more widespread in wild animals in our country and in Europe (12, 13, 20, 22).

Blaga, R. et al. (2009) reported the highest prevalence of *Trichinella* infection in wolves, European wild cats, and red foxes. The geographical distribution of *Trichinella* spp. in Romania is not specific, with both species being present in all the counties studied (3).

The samples collected from wild boar populations (*Sus scrofa*) from Bihor County, Romania, were examined by the method of artificial digestion, ELISA, and Western Blot. The results of serological examinations revealed the existence of the nematode *Trichinella spp.* in the wild boar in this area of our country. Moreover, the authors warn people who prefer to consume wild boar meat as a source of infestation with larvae of *Trichinella* spp. even when they are found in low numbers in infested meat (5).

Another study describes the presence of *Trichinella britovi* larvae in wild boars from Valcea County, Romania (17).

Also, in our country, the study conducted by Nicorescu et al., 2015 reveals the highest prevalence of *Trichinella* infestations in bears, followed by wild boars and domestic pigs. Infestations with the species *T. spiralis*, *T. britovi*, but also mixed infestations with *T. spiralis* and *T. britovi* were identified (19).

Molecular studies carried out in Croatia over a period of eight years revealed the presence of four species of *Trichinella* in the samples collected from wild boars: *T. spiralis, T. pseudospiralis, T. britovi, T. nativa* (1).

The present study describes the epidemiological evolution of trichinellosis in wild boars over five years (2017-2021). Throughout the evaluated period, cases of wild boar infestation have been registered annually, an aspect that alerts human and veterinary specialists, hunters, and people who consume game meat.

Conclusions

The annual trichinellosis diagnosis in wild boars, in Maramures County over a period of 4 years, warns the human consumer, especially hunters, to the importance of game meat control and alerts the veterinarian specialists about the parasitological control of the disease in this area of Romania.

References

1. **Balic, D., Marucci, G., Agicic, M., Benic, M.,Krovina, Z., Miskic, T., Aladic, K., Skrivanko, M.,** *Trichinella spp.* in wild boar (Sus scrofa) populations in Croatia during an eight-year study (2010-2017), One Health, 2020, 11, 100172.
2. **Bilska-Zając, E., Różycki, M., Grądziel-Krukowska, K., Bełcik, A., Mizak, I., Karamon, J., Sroka, J., Zdybel, J., Cencek, T.,** Diversity of *Trichinella* species in relation to the host species and geographical location, Veterinary Parasitology, 2020, 279, 109052.
3. **Blaga, R., Gherman, C., Cozma, V., Zocevic, A., Pozio, E., Boireau, P.,** *Trichinella* species circulating among wild and domestic animals in Romania, Veterinary Parasitology, 2009, 159, 3-4, 218-21.
4. **Boros, Z., Ionică, A.M., Deak, G., Mihalca, A.D., Chişamera, G.B., Gyorke, A., Gherman, C., Cozma, V.,** The European badger, *Meles meles*, as a new host for *Trichinella britovi* in Romania, Veterinary Parasitology, 2021, 297, 109545.
5. **Boros, Z., Vallee, I., Panait, L.C., Gherman, C.M., Chevillot, A., Boireau, P., Cozma, V.,** Seroprevalance of *Trichinella spp.* in wild boars (*Sus scrofa*) from Bihor county, western Romania, Helmintologia, 2020, 57, 3, 235-240.
6. **Bruschi, F., Pozio, E.,** *Trichinella britovi*, Trends in Parasitology, 2020, 36, 2, 227-228.
7. **Ciupa, A., Mihaiu, M., Blaga, R., Dan, S.D.,** Optimization method for biomolecular identification of muscle larva of *Trichinella* species, Veterinary Medicine, 2010, 67, 1, 374-379.

8. Commission Implementing Regulation (EU) 2015/1375 of 10 August 2015 laying down specific rules on official controls for *Trichinella* in meat (codification).
9. **Dărăbuș, Gh., Oprescu, I., Morariu, S., Mederle, N., Ilie, M., Imre, M.,** Parazitologie și Boli Parazitare, Ed. Mirton, Timișoara, 2022.
10. **Di Salvo, A.R., Chomel, B.B.,** Zoonoses and potential zoonoses of bears, Zoonoses and Public Health, 2020, 67, 1, 3-13.
11. **Diaz, J.H., Warren, R.J., Oster, M.J.,** The Disease Ecology, Epidemiology, Clinical Manifestations, and Management of Trichinellosis Linked to Consumption of Wild Animal Meat, Wilderness & Environmental Medicine, 2020, 31, 2, 235-244.
12. **Gherman, C.M., Boros, Z., Băieș, M.H., Cozma-Petruț, A., Cozma V.,** A review of *Trichinella* species infection in wild animals in Romania, Food Waterborne Parasitology, 2022, 28:e00178.
13. **Gómez-Morales, M.A., Ludovisi, A., Amati, M., Cherchi, S., Tonanzi, D., Pozio, E.,** Differentiation of *Trichinella* species (*Trichinella spiralis*/ *Trichinella britovi versus Trichinella pseudospiralis*) using western blot, Parasites & Vectors, 2018, 11, 1, 631.
14. **Marian, I., Mihalca, A.D., Gherman, C.M.,** Prevalence of *Trichinella* spp. infection in large wild carnivore species from Romania between Jan 2014 and July 2015, BUASVM, 2015, 72, 2, 438-440.
15. **Marin A.M., Popovici D.C., Pavlovic I., Ilie M.S., Moraru M.M.F., Ghilean B., Dreghiciu I.C., Mederle N.,** Study on the prevalence and larval burden of the nematode *Trichinella* spp. in jackals from hunting grounds in Timis County (Romania), Lucrări Științifice Medicină Veterinară Timișoara, 2022, LV(4), 105-111.
16. **Marin, A.M., Dărăbuș Gh., Herman, V., Morariu, S., Olariu, T.R., Dărăbuș, R.G., Sîrbu, B., Mederle, N.,** Study on the prevalence and larval burden of the nematode *Trichinella spp*. in red foxes from hunting grounds in Timis county, Revista Română de Medicină Veterinară, 2021, 31, 2, 23-26.
17. **Marin, A.M., Oprescu, I., Dărăbuş, G., Imre, M., Hădărugă, N., Moraru, M.M.F., Ghilean, B.M., Mederle, N.,** Updates on *Trichinella* species infection in wild boar (*Sus scrofa*) from Southern Romania, Journal of Agroalimentary Processes and Technologies, 2022, 28, 4, 388-393.
18. **Moraru, M.M.F., Herman, V., Oprescu, I., Fodor, J.T., Morariu, S., Ilie M., Marin, A.M., Mederle, N.,** Wild boar - The source of human contamination with T*richinella* spp. in Romania, Revista Română de Medicină Veterinară, 2022, 32, 2, 38-42.
19. **Nicorescu, I.M., Ionita, M., Ciupescu, L., Buzatu, C.V., Tanasuică, R., Mitrea, I.L.,** New insights into the molecular epidemiology of *Trichinella* infection in domestic pigs, wild boars, and bears in Romania, Veterinary Parasitology, 2015, 212, 3-4, 257-61.

20. **Pavic, S., Andric, A., Sofronic-Milosavljevic, L.J., Gnjatovic, M., Mitic, I., Vasilev, S., Sparic, R., Pavic, A.,** *Trichinella britovi* outbreak: Epidemiological, clinical, and biological features, Medicine et Maladies Infectieuses, 2020, 50, 6, 520-524.
21. **Pozio E., Zarlenga D.,** International Commission on Trichinellosis: Recommendations for genotyping *Trichinella* muscle stage larvae, Food and Waterborne Parasitology, 2019, 15, e00033.
22. **Pozio, E., Rinaldi, L., Marucci, G., Musella, V., Galati, F., Cringoli, G., Boireau, P., La Rosa, G.,** Hosts and habitats of *Trichinella spiralis* and *Trichinella britovi* in Europe, International Journal of Parasitology, 2009, 39, 1, 71-9.
23. **Pozio, E.,** Scientific achievements of the last 60 years: From a single to a multispecies concept of the genus *Trichinella*, Veterinary Parasitology, 2020, 30, 109042.
24. **Pozio, E., Zarlenga, D.S.,** Recent advances on the taxonomy, systematics and epidemiology of *Trichinella,* International Journal of Parasitology, 2005, 35, 11-12, 1191-1204.
25. **Zarlenga, D., Thompson, P., Pozio, E.,** *Trichinella spp*. and genotypes, Research in Veterinary Science, 2020, 133, 289-296.
26. ***http://www.ansvsa.ro/download/man.oper_.crize_.alimentare/Manual-operational-aplicabil-in-cazul-confirmarii-*Trichinella*-spp.pdf

BONE MARROW CORE SAMPLES. COMPARATIVE STUDY BETWEEN ILIAC AND HUMERAL BIOPSIES IN DOGS

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Summary

Bone marrow evaluation in veterinary medicine is less used due to the high costs, but also due to the need of special knowledge and specific techniques. The literature in the field points out the importance of evaluating the bone marrow, showing its implications both in the field of internal medicine and oncology, but also in the field of infectious diseases and parasitology. The purpose of this study is to determine if there are differences between bone marrow samples collected from the iliac site and humeral site. The specimens were collected from 14 dogs shortly after death using a 13 G Jamshidi needle. Samples were collected from the dorsal iliac crest and from the great tubercle of the humerus from each dog. The histopathological assessment was done using the parameters proposed by Abrams Ogg et al. in 2012, thus, each biopsy received a quality score based on the number of intra-trabecular spaces free of artifact. Other parameters taken in consideration were the cell density, megakaryocyte density and the presence or absence of iron stores. Results show that the iliac biopsies had a better-quality score (mean score – 2.85) compared to the humeral biopsies (2.07). 12/28 biopsies had an acceptable score, with 9/12 being collected from the iliac site. In most of the samples (11/14), there were no significant differences between the iliac and humeral samples. Major differences were recorded in 3/14 cases involving the megakaryocyte density. Differences were corelated with a lower biopsy score. There were no significant differences regarding the cell density and iron stores. High quality samples are essential for an accurate evaluation of the bone marrow. Although the iliac samples were of higher quality, humeral biopsy remains a preferred site, being more accessible, especially in small animals considering the sizes of the biopsy needles, which are designed for human use.

Keywords: bone marrow, core biopsy, histopathology, veterinary hematology.

Evaluation of bone marrow is advised when peripheral blood disturbances are observed (8). Such disturbances include some types of anemias, leukopenia, or thrombocytopenia without regeneration.

Bone marrow examination is also recommended when there are atypical blood cells observed in the peripheral blood smear, but also when occult neoplasia or infection is suspected (5). One study suggests that bone marrow examination is also a practical method for diagnostic of leishmaniosis in dogs (11). When considering bone marrow examination, the decision must be made based on some variables. In correlation with the clinical aspects and other paraclinical findings, such variables include the age of the animal, the presence of hematological disturbances, the presence of single or multi-lineage dysplasia or the presence of blast cells in peripheral blood (10).

Bone marrow samples cand be either an aspirate or a core biopsy. By using the aspiration method alone, bone marrow architecture cannot be assessed (9). Also, overall cellularity and focal changes can be missed by evaluating only aspiration smears (12). Bone marrow cores are collected for histopathological examination and may provide information about structural organization of the hematopoietic tissue, bone, interstitial space, and adipose tissue (13). Inflammation, neoplasia, necrosis, or fibrosis are also observed on core samples (12). Also, there are situations when aspirations are nondiagnostic (dry taps). In such cases, a core sample is indicated (14). Bone marrow histopathology can also identify focal infiltrates of lymphoid cells that may be dispersed on cytological smears (8). In cases of myelofibrosis, only histopathological examination of the bone marrow can provide a definitive diagnosis (8). Although the term “biopsy” refers to samples collected from living animals, this study uses samples collected post-mortem and are referred as “biopsies” because the protocol and technique for collection are the same. An accurate diagnosis of the bone marrow implies quality specimens; thus, it is important to pay attention while collecting and processing the samples (18). Furthermore, bone marrow examination is not an invasive procedure and associated complications are rare (20).

The literature points out the anatomical sites for bone marrow sampling: the proximal humerus, the iliac crest, the proximal femur, the sternum, and the ribs (19). For core biopsies, the proximal humerus and the iliac crest are the most common sites. The purpose of this study is to determine if there are differences regarding the quality of the specimens between bone marrow samples collected from the iliac site and humeral site.

Materials and methods

The specimens were collected from 14 dogs shortly after death during routine post-mortem examination. The dogs were medium to large, aged between 2 and 15 years, from which 8 were males and 6 females (Table 1). Samples were collected from the dorsal iliac crest and from the great tubercle of the humerus from each dog using a 13G manually operated Jamshidi type needle, resulting a total of 28 samples.

These sites were selected because they are the most accessible and most frequently used for core biopsies. For the iliac sample, the animal was placed in sternal recumbency, and the hind limbs were positioned in full flexion having the paws placed cranially under the axillar region. In overweight animals, palpating the iliac crest is very difficult (19). If the dogs were large, they were placed in lateral recumbency (Fig. 2). For the humeral site, the animal was placed in lateral recumbency. Before inserting the needle perpendicular to the length of the humerus, the great tubercle was palpated (19). Gross examination of the specimens was done to check adequate samples (Fig. 1). Cortical bone appears white, having a fine appearance, while normal bone marrow appears red (4). Following collection,

samples were fixed and decalcified, embedded in paraffin and histological slides were obtained. The histological assessment was done using the parameters proposed by Abrams Ogg et al. (1) (Table 2), thus, each biopsy received a quality score based on the number of inter-trabecular spaces free of artifact. Other parameters taken in consideration were the cell density, megakaryocyte density and the presence or absence of iron stores.

Table 1

Cases used in this study

No.	Breed	Age	Sex*
1	Mix-breed	6 years	M.N.
2	Mix-breed	14 years	F.S.
3	Mix-breed	6 years	M.N
4	Mix-breed	5 years	M.I.
5	Golden Retriever	2 years	M.I
6	German Shepard	5 years	F.I.
7	Mix-breed	14 years	F.I.
8	Chow-Chow	10 years	F.S.
9	Bull mastiff	6 years	F.S.
10	Rottweiler	11 years	F.S.
11	Mix-breed	10 years	M.I.
12	German Shepard	3 years	M.N.
13	Mix-breed	6 years	M.N.
14	Mix-breed	15 years	M.N.

*M.N. – male, neutered; M.I. – male, intact; F.S. – female, spayed; F.I. – female, intact

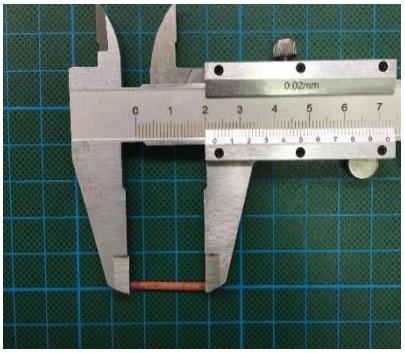

Fig. 1. Adequate bone marrow specimen – around 2 cm. length core sample showing normal bone marrow (red) (original).

Fig. 2. Sampling from the iliac crest. The dog was placed in lateral recumbency. The area was clipped, and the needle was inserted through a small stab

Table 2

Histologic criteria for bone marrow evaluation (1, 2)

	Number of intertrabecular spaces free of artifact				
	0	1-2	3	4-5	≥6
Sample quality score	1	2	3	4	5
Cell density	Low		Medium	High	
	Number of megakaryocytes per 10x field				
Megakaryocyte density	<3		4-9	≥10	
	Low		Medium	High	
Iron stores	Absent		Present		

Results and discussions

Results show that the iliac biopsies had a better-quality score (mean score – 2.85) compared to the humeral biopsies (2.07), based on the number of intertrabecular spaces free of artifact (Fig. 4). The most common artifacts were blood contamination (due to collection technique) and loss of hematopoietic cells. 12/28 biopsies had an acceptable score (at least 3), with 9 of them being collected from the iliac site. In most of the samples (11/14), there were no significant differences between the iliac and humeral samples.

Megakaryocyte density was estimated from counting in non-overlapping 10x fields and iron stores were noted absent or present, if observed as brown pigment in H&E sections (2) (Fig. 3, 5 - 9). Major differences were recorded in 3/14 cases involving the megakaryocyte density. Differences were corelated with a lower biopsy score. There were no significant differences regarding the cell density and iron stores. Most of the samples showed medium cell density (Fig. 3, 5 - 9). Only one sample showed low cell density and was also the lowest-scoring sample (Table 3). A maximum of two attempts were necessary for adequate samples from each site, but overall, it was easier to obtain a sample from the humerus.

This study has some limitations, such as the fact that it was conducted on non-living animals. Also, the quality of the samples may be affected by the harvesting technique, and by the type of needle that is used for sampling.

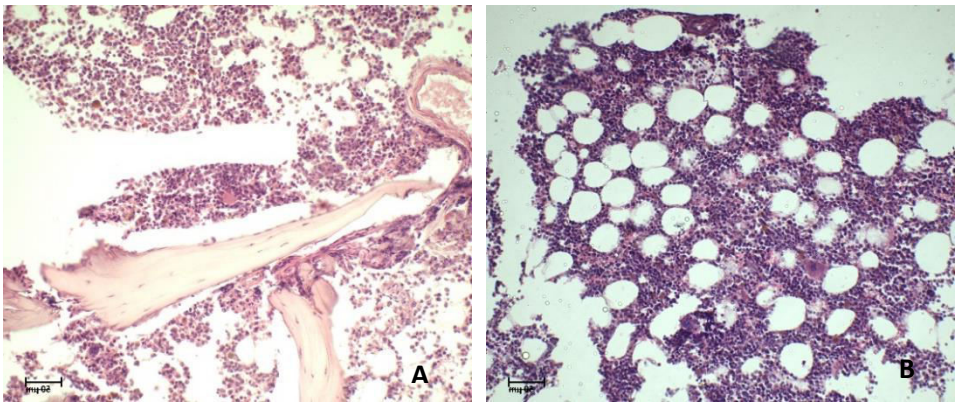

Fig. 3. A - Bone marrow section from case no. 11 – humeral sample - intertrabecular spaces showing bone trabeculae and medium cell density, 200x, H.E.;
B - Bone marrow section from case no. 6 – iliac sample - intertrabecular space free of artifact with medium cell density and medium megakaryocyte density, 200x, H.E (original)

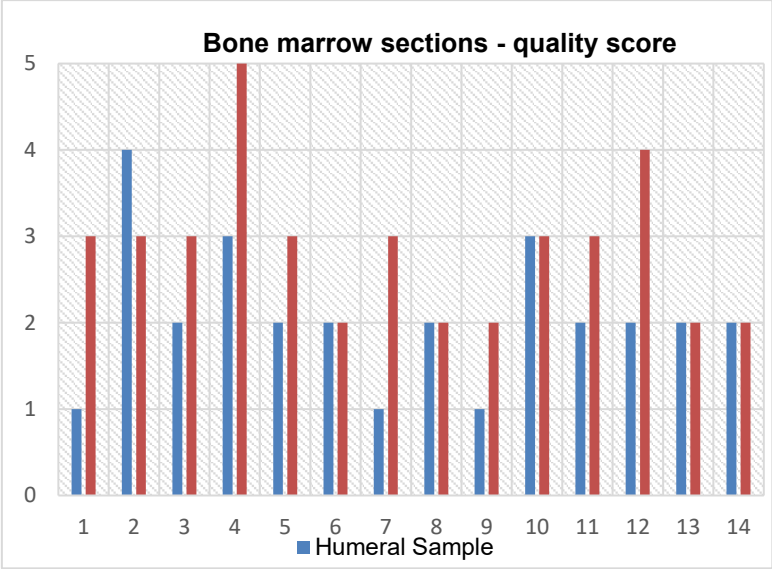

Fig. 4. Graphic representation of bone marrow quality score for each sample: vertical axis – case number; horizontal axis – sample quality score

One recent study that analyzed 280 bone marrow samples collected using two different manually operated Jamshidi type needles, suggests that the biopsy needle type can affect the quality of the specimen (3).

Another limitation of this study is that the myeloid to erythroid ratio was not evaluated. A recent study suggests that bone marrow precursors may not be distributed uniformly throughout the collection sites for bone marrow, which can lead to differences in evaluating the myeloid to erythroid ratio (7). Another study suggests that sampling bone marrow core with a 15G needle is easy to perform in small dogs when collecting from the humerus and provides better quality samples than samples collected from the iliac site. However, samples collected with the 15G needle were more likely to receive a lower score than those collected with the 13G needle (2).

Table 3

Results from histologic evaluation

No		Number of intertrabecular spaces free of artifact	Biopsy quality score	Cell density	Cell density %	Number of Megakaryo-cyte/100x field	Megakaryo-cyte density	Iron Stores
1	H	0	1	low	10%	0	low	+
	I	3	3	med	50%	2	low	+
2	H	4	4	med	40%	3	low	+
	I	3	3	med	40%	4	med	+
3	H	1	2	med	40%	1	low	+
	I	3	3	med	30%	3	low	+
4	H	3	3	med	30%	2	low	+
	I	>6	5	med	40%	3	low	+
5	H	2	2	med	60%	15	high	+
	I	3	3	med	40%	8	med	+
6	H	2	2	med	60%	5	med	+
	I	2	2	med	70%	5	med	+
7	H	0	1	med	30%	3	low	+
	I	3	3	med	30%	3	low	+
8	H	2	2	med	50%	5	med	+
	I	1	2	med	50%	4	med	+
9	H	0	1	med	40%	4	med	+
	I	2	2	med	40%	6	med	+
10	H	3	3	med	60%	3	low	+
	I	3	3	med	60%	2	low	+
11	H	2	2	med	60%	8	med	+
	I	3	3	med	60%	3	low	+
12	H	2	2	med	30%	2	low	+
	I	4	4	med	30%	2	low	+
13	H	1	2	med	50%	5	med	-
	I	1	2	med	50%	3	low	+
14	H	2	2	med	40%	2	low	+
	I	1	2	med	50%	3	low	+

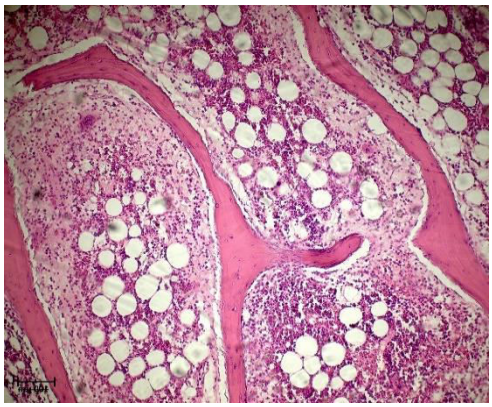

Fig. 5. Bone marrow section from case no. 4 – iliac sample - intertrabecular spaces free of artifact with medium cell density and low megakaryocyte density, 100x, H.E.

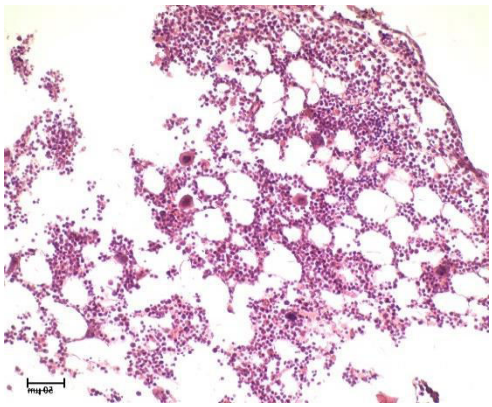

Fig. 6. Bone marrow section from case no. 5 – humeral sample - intertrabecular space free of artifact with high megakaryocyte density, 200x, H.E.

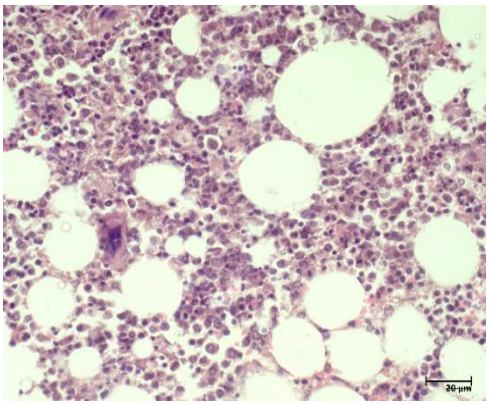

Fig. 7. Bone marrow section from case no. 10 – humeral sample - intertrabecular space free of artifact with medium cell density and 2 megakaryocytes, 400x, H.E.

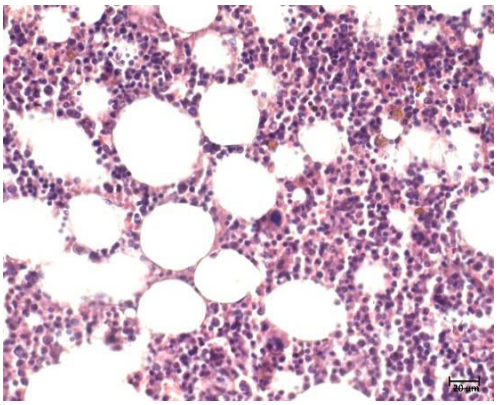

Fig. 8. Bone marrow section from case no. 6 – iliac sample - intertrabecular space free of artifact with medium cell density and iron stores (upper right), 400x, H.E.

A similar study performed on cats concluded that high quality specimens can be obtained from the humerus using a 15G needle. Also, good quality specimens can be obtained from cats from the ilium using a 13G needle (1). The same authors suggest that overall, quality bone marrow samples from cats remains challenging, no matter the type of the needle, with only 50% of the samples having an acceptable score (1). One study compared bone marrow samples collected with the manual technique with samples collected with a rotary battery-powered device in dogs and

cats and suggests that the rotary battery-powered bone marrow collection system can provide larger sized specimens which implies a greater chance of having high quality samples, and, samples were easier to obtain (17).

Bone marrow examination should be used more often as it is a useful tool for diagnosis of hematopoietic disorders, but not only. Bone marrow histopathology can be used in cases of hyperproteinemia or hypercalcemia associated with multiple myeloma or lymphoma with bone marrow involvement (8). It also provides diagnosis when looking for metastatic neoplasia in the bone marrow (8). Some carcinomas, such as those that originate in the prostate, mammary and lungs can metastasize to the bone marrow, most frequently in the axial skeleton and proximal long bones (8). Histopathology is essential for an accurate diagnosis of such lesions. Thus, a high-quality sample that has more intertrabecular spaces free of artifact means a greater chance for an accurate evaluation of bone marrow, but also an increased chance for detecting focal lesions.

Conclusions

High quality samples are essential for an accurate evaluation and diagnostic of bone marrow. This study shows that the iliac samples tend to have higher quality, but the iliac site for collection is not always approachable as the needles used for sampling are designed for human use, thus, iliac samples from small breed dogs are very difficult to obtain. Also, in obese animals, the humeral site is much more approachable than the iliac site. Therefore, if accessible, the iliac site should be approached first for bone marrow core samples, but overall, the humeral site remains the preferred one.

References

1. **Abrams-Ogg, A.C.G., Defarges, A., Bienzle, D.,** Comparison of feline core bone marrow biopsies from different sites using 2 techniques and needles, Veterinary Clinical Pathology, 2014, 43/1, 36-42.
2. **Abrams-Ogg, A.C.G., Defarges, A., Foster R.A., Bienzle, D.,** Comparison of canine core bone marrow biopsies from multiple sites using different techniques and needles, Veterinary Clinical Pathology, 2012, 41, 235-242.
3. **Brestoff, J.R., Toland, A., Afaneh, K., Qavi, A.J., Press, B., Westervelt, P., Kreisel, F., Hassan, A.,** Bone Marrow Biopsy Needle Type Affects Core Biopsy Specimen Length and Quality and Aspirate Hemodilution, American Journal of Clinical Pathology, 2018, XX, 1-9.
4. **Byers, C.G.,** Diagnostic bone marrow sampling in cats. Currently accepted best practices, Journal of Feline Medicine and Surgery, 2017, 19, 759-767.
5. **Defarges, A., Abrams-Ogg, A.C.G., Foster, R.A., Bienzle, D.,** Comparison of sternal, iliac, and humeral bone marrow aspiration in Beagle dogs, Veterinary Clinical Pathology, 2013, 1-7.

6. **Ettinger, J.S., Feldman, E.C., Côté, E.,** Textbook of Veterinary Internal Medicine, 8th Edition, Elsevier, 2017.
7. **Gal, A., Burchell, R.K., Worth, A.J., Lopez-Villallobos, N., Marshall J.C., MacNeill, A.L.,** The Site of Bone Marrow Acquisition Affects the Myeloid to Erythroid Ratio in Apparently Healthy Dogs, Veterinary Pathology, 2018, 55, 6, 853-860.
8. **Harvey, J.W.,** Veterinary Hematology. A Diagnostic Guide and Color Atlas, Elsevier, 2012.
9. **Mathis, J.C., Seung, H.Y., Lauren, A.S.,** Diagnosis of secondary dysmyelopoiesis via costochondral rib aspirate in a dog, Journal of Veterinary Emergency and Critical Care, 2014, 24, 6, 739-44.
10. **Mylonakis, M., Hatzis, A.,** Practical bone marrow cytology in the dog and cat, Journal of the Hellenic Veterinary Medical Society, 2017, 65, 3, 181-196.
11. **Paparcone, R., Fiorentino, E., Cappiello, S., Gizzarelli, M., Gradoni, L., Oliva, G., Foglia Manzillo, V.,** Sternal Aspiration of Bone Marrow in Dogs: A Practical Approach for Canine Leishmaniasis Diagnosis and Monitoring, Journal of Veterinary Medicine, 2013, 2013, 217314.
12. **Raskin, R.E., Messick, J.B.,** Bone Marrow Cytologic and Histologic Biopsies: Indications, Technique, and Evaluation, Veterinary Clinics: Small Animal Practice, 2012, 42, 23-42.
13. **Reagan, W.J., Irizarry-Rovira, A., Poitout-Belissent, F., Bolliger, A.P., Ramaiah, S.K., Travlos, G., Walker, D., Bounous, D., Walter, G.,** Best Practices for Evaluation of Bone Marrow in Nonclinical Toxicity Studies, Toxicologic Pathology, 2011, 39, 435-448.
14. **Reeder, J.P., Hawkins, E.C., Cora, C.M., Marks, S.L., Grindem, C.B.,** Effect of a Combined Aspiration and Core Biopsy Technique on Quality of Core Bone Marrow Specimens, American Animal Hospital Association, 2013, 49, 16-22.
15. **Roger, S.R., Hogan, T.F., Pavot, D.R., Forysthe, R., Massey, D., Smith, E., Wright, L.Jr., Ben-Ezra, J.M.,** A Pathologist's Perspective on Bone Marrow Aspiration and Biopsy: I. Performing a Bone Marrow Examination, Journal of Clinical Laboratory Analysis, 2004, 18, 70-90.
16. **Silva do Nascimentoa, J., Maues, T., Leiteb, J.S., Ferreirab, A.M.R., Ferreira, M.L.G.,** Core-Needle Biopsy Efficacy in Histopathology Diagnosis of Canine Multicentric Lymphoma, Topics in Companion An Med, 2021, 45, 100561.
17. **Tappin, S.W., Lorek, A., Villiers E.J.,** Use of a rotary battery-powered device for the collection of bone marrow in dogs and cats, Veterinary Record 2014, 175, 173.
18. **Travlos, G.S.,** Histopathology of Bone Marrow, Toxicologic Pathology, 2006, 34, 566-598.
19. **Townsend, F.I.,** Bone marrow aspiration in dogs and cats, Lab animal, 2008, 37, 11, 497.

20. **Woods, G.A., Simpson, M., Boag, A., Paris, J., Piccinelli, C., Breheny, C.,** Complications associated with bone marrow sampling in dogs and cats, Journal of Small Animal Practice, 2020, 1-7.

ECHOSTRUCTURE AND ECHOANATOMY DATA OF ULTRASONOGRAPHIC EXAMINATION IN ALPACAS (*VICUGNA PACOS*)

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Summary

The ultrasound examination, due to the relevance of the information obtained regarding the topography, size/volume, echostructure and echogenicity of the examined structures, is among the most accessible imaging diagnostic methods in field conditions in animals. Due to the increase in popularity of this species in Romania in the last decade, it is opportune to enrich the literature data for the veterinarian in order to optimally manage organopathies and systemic diseases in alpacas. The study was carried out over a period of 2 years 2020 - 2022, on a number of 6 alpacas from the Bucharest Zoo and, respectively, some private leisure properties in Bucharest. Animals are integrated into the leisure service as well as therapy animals. An ESAOTE FALCO 100 ultrasound machine with a 5 MHz linear probe and a 5 MHz convex probe was used in the study. The ultrasound examination of the 3 gastric compartments was possible and easy respecting the eco-anatomical approach characteristic of the species, providing imaging data in real time regarding the topography, the degree of distention, the type and quantity of the content, the motility and thickness and the preservation of the characteristic parietal architecture. Ultrasonographic evaluations of the small intestine and the large intestine were possible, allowing to obtain information on the characteristic tone and peristalsis, along with the highlighting of the parietal components for the small intestine and more difficult for the large intestine, where the presence of gases artifactually limits the identification and appreciation of the distal wall of intestinal contents (gas). The examination of the spleen was possible following the approach in the area of choice (at the level of the left hypochondrium), obtaining information regarding the size, echostructure and echogenicity of the lienal parenchyma, in the patients evaluated clinically and sonographically, no changes in the characteristic parameters were recorded.

Keywords:ultrasonography, *Alpaca, Vicugna pacos.*

The ultrasound examination, due to the relevance of the information obtained regarding the topography, size/volume, ecostructure and echogenicity of the examined structures, is among the most accessible imaging diagnostic methods in field conditions in animals (6).

Due to the increase in popularity of this species in Romania in the last decade, it is opportune to enrich the literature data for the veterinarian in order to optimally manage organopathies and systemic diseases in alpacas.

Materials and methods

The study was carried out over a period of 2 years 2020 - 2022, on a number of 6 alpacas from the Bucharest Zoo and, respectively, some private leisure properties in Bucharest. Animals are integrated into the leisure service as well as therapy animals. An ESAOTE FALCO 100 ultrasound machine with a 5 MHz linear probe and a 5 MHz convex probe was used in the study (Fig. 1, Fig. 2).

Fig. 1. Esaote Falco 100 ultrasound

Fig. 2. Linear probe 5 MHz

Table 1

Description of the patients in this article

	NUMBER OF ALPACAS (n=6)			
BREED		**AGE**		**SEX**
		1-5 years	**6-10 years**	**M/F**
Suri	***(n=2)***			
Huacaya	***(n=4)***	***(n=4)***	***(n=2)***	**F=2** **M=4**

Fig. 3. Ultrasound examination in the quadrupedal position

Results and discussions

Gastrointestinal tract

The ultrasound examination can begin by scanning the right abdomen around the tenth intercostal space, approximately 5 cm ventral to the origin of the rib. At this level, the ultrasound will cross caudo-dorsally the lung, the diaphragm and the medio-dorsal portion of the liver, and if the probe is oriented slightly ventrally, the liver can be captured. If the probe is gradually moved ventrally, following the intercostal space along the right lobe of the liver, both the duodenum and the dorsal portion of C-3 can be captured, near the area where the glandular and non-glandular regions of this compartment converge (5, 7, 16) (Fig. 3).

If the probe is moved in the caudo-cranial direction approximately 10-20 cm from C-3, the triangle formed by the liver, duodenum and C3 can be followed, evaluated to see if there are traces of ulcers at this level. For the right abdomen one will start from the level of the ninth and eleventh intercostal space to the right paramedian region to visualize the duodenum, pylorus and pyloric antrum. It will be possible to continue in the same plan for the ultrasound image of the small intestine (3, 8, 11).

In the right paralumbar area, images of the ascending colon and large intestine can be captured. Small bowel segments have a hypoechoic appearance of intraluminal contents and increased motility compared to large bowel segments (2, 9, 13).

The ventral, cranial and caudal portions of the first compartment can be captured in the left ventral area of the abdomen. Compartment motility can be assessed, due to glandular sacs and compartment orientation (1, 17).

Kidneys

Both kidneys can be visualized at the level of the paralumbar fossa, ventral to the transverse processes of the lumbar vertebrae. The kidneys are located near the dorso-median line of the abdomen, the left kidney being positioned slightly caudal to the right kidney (12, 15) (Fig. 4, Fig. 5).

It is recommended that ultrasound evaluation begin with the right kidney at the level of the right paravertebral fossa, approximately at the level of the fifth or seventh transverse lumbar process (10, 19).

Camelid kidneys are not lobulated, but have a smooth capsule that incorporates the renal pelvis, medullary and cortical. The capsule may appear hyperechoic normally and should not be surrounded by fluid or gas. The renal pelvis may also appear hyperechoic, and sometimes clear anechoic sacs diffuse from the renal pelvis into the medulla. By placing the probe ventrally, to the left of the transverse lumbar process, the left kidney can be captured in the image. The spleen lies cranial to the left kidney. The two kidneys have the same dimensions, respectively 5-7 cm (14, 20).

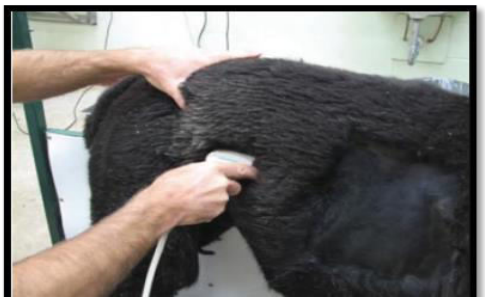

Fig. 4. Positioning of the ultrasound probe to image the right kidney (9)

Fig. 5. Positioning of the ultrasound probe to image the left kidney (9)

Urinary bladder

For the ultrasound capture of the urinary bladder, the probe must be placed caudo-ventrally at the level of the flank, and then it will be oriented caudo-dorsally towards the pelvis. The normal bladder should not be more than 6-8 cm in diameter in adults. Hyperechogenic irregularities can be observed suspended in the bladder lumen in healthy patients and are not pathognomonic for pathologies evolving at this level. Transrectal ultrasound examination provides detailed images of the bladder, pelvic urethra, and male gonads (4, 18).

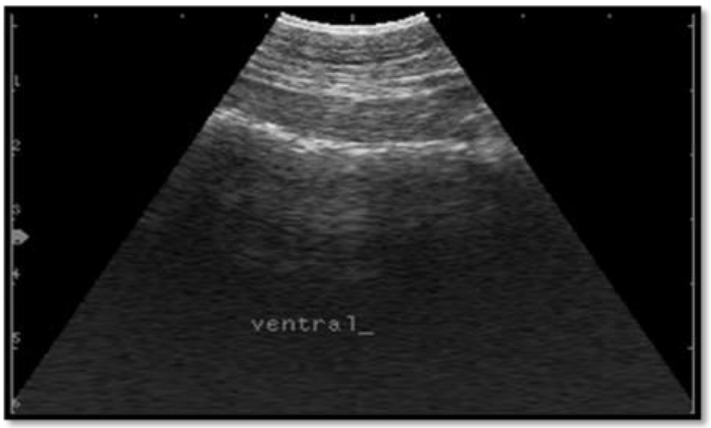

Fig. 6. C1 image taken with a convex probe with a 5 MHz transducer

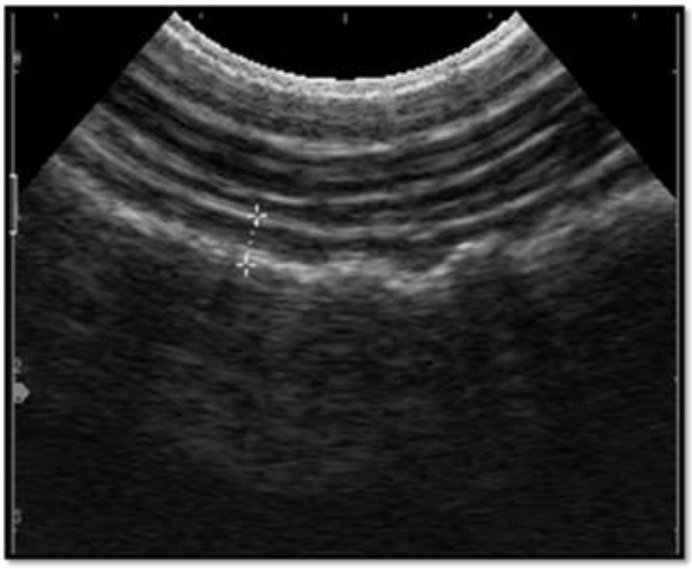

Fig. 7. C1 image taken with a convex probe with a 5 MHz transducer

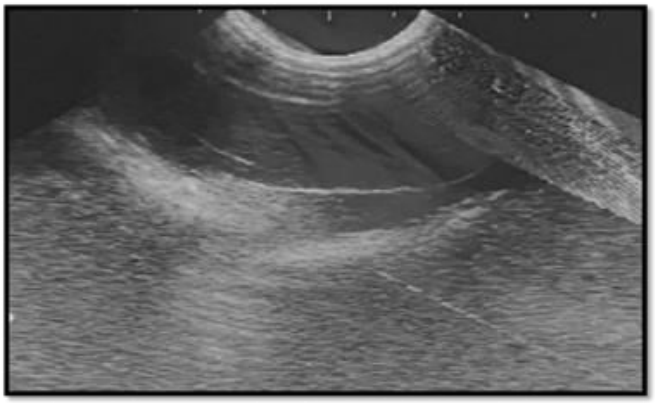

Fig. 8. Image C3, the area of convergence between the glandular region and the non-glandular region

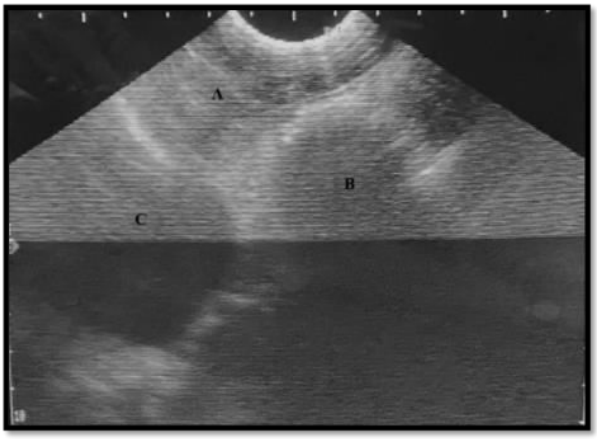

Fig. 9. A. First compartment, B. Second compartment, C. Third compartment

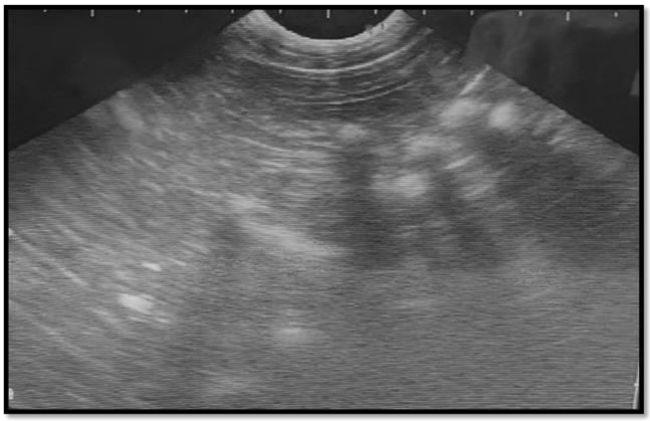

Fig. 10. Longitudinal large intestine image, made with a convex probe with a frequency of 5 MHz

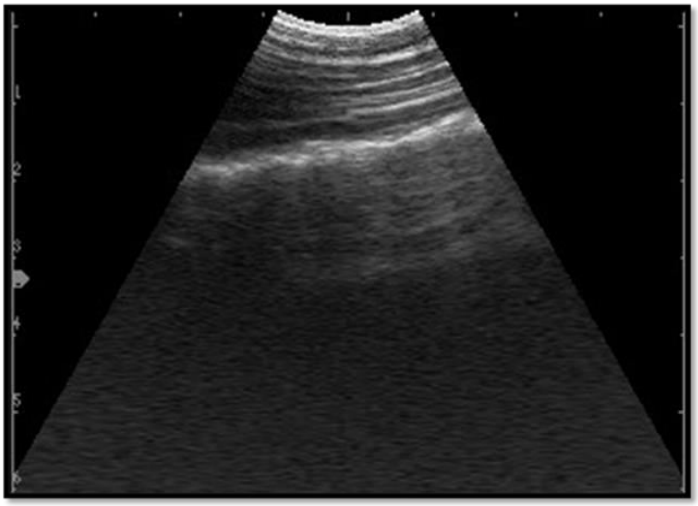

Fig. 11. Normal-appearing small intestine in oblique longitudinal section

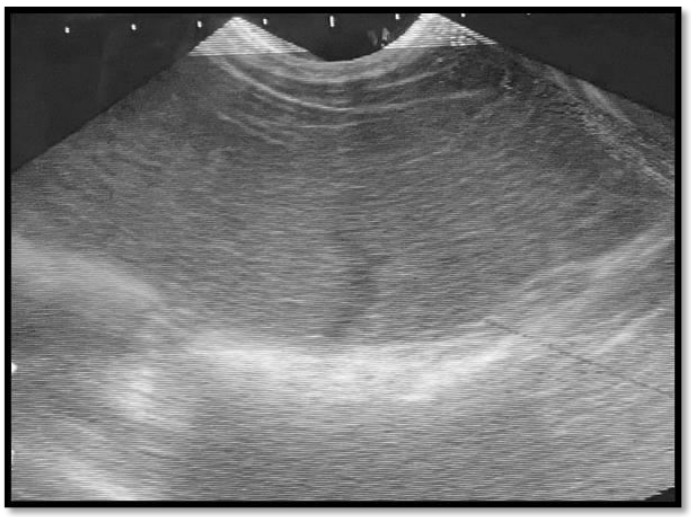

Fig. 12. Spleen with normal appearance in ventro-dorsal incidence

The ultrasound examination of the 3 gastric compartments was possible and easy respecting the eco-anatomical approach characteristic of the species, providing imaging data in real time regarding the topography, the degree of distention, the type and quantity of the content, the motility and thickness and the preservation of the characteristic parietal architecture (Fig. 6, Fig. 7, Fig. 8, Fig. 9).

Ultrasonographic evaluations of the small intestine (Fig. 11) and the large intestine (Fig. 10) were possible, allowing to obtain information on the characteristic tone and peristalsis, along with the highlighting of the parietal components for the small intestine and more difficult for the large intestine, where the presence of gases artifactually limits the identification and appreciation of the distal wall of intestinal contents (gas).

The examination of the spleen (Fig. 12) was possible following the approach in the area of choice (at the level of the left hypochondrium), obtaining information regarding the size, echostructure and echogenicity of the lienal parenchyma, in the patients evaluated clinically and sonographically, no changes in the characteristic parameters were recorded.

Ultrasound investigations of the kidneys allowed obtaining imaging information in real time, allowing obtaining ultrasound coordinates regarding the topography, volume, echostructure and echogenicity of the renal parenchyma, which would complete the diagnostic protocol in this species.

Conclusions

The opportunity to implement a diagnostic protocol using the ultrasonographic examination in abdominal pathology in alpacas is revealed by the early and noninvasive diagnosis and the lack of dramatic clinical manifestations in this species.

The discrete changes that can be identified by the ultrasonographic examination underline the accuracy and high specificity of the imaging method.

References

1. **Aiello, S.M.,** The Merck Veterinary Manual, 11th Edition, Wiley, 2017.
2. **Anderson, D.E.,** Veterinary techniques for llamas and alpacas, Iowa: Wiley-Blackwell, 2013.
3. **Cebra, C.A.,** Llama and alpaca care: Medicine, Surgery, Reproduction, Nutrition, and Herd Health, St. Louis: Elsevier, 2014.
4. **Codreanu, M.D.,** Tratat de ultrasonografie clinică veterinară, Editură Coral Sanivet, Bucureşti, 2022.
5. **Colville, T.P.,** Clinical anatomy and physiology for veterinary technicians, Elsevier - Health Sciences Division, 2016.
6. **DesCôteaux, L.G.,** Practical atlas of ruminant and camelid reproductive ultrasonography, Iowa: Wiley-Blackwell, 2010.
7. **Dulphy, J.P.,** Comparative study of forestomach digestion in llamas and sheep. Reproduction, Nutrition, Development, 1977.
8. **Eibl, C.,** Ultrasonography of kidney and spleen in clinically healthy llamas and alpacas, Acta veterinaria Scandinavica, 2021, 63, 1, 4.
9. **Farrow, C.S.,** Veterinary diagnostic imaging birds, Exotic Pets, and Wildlife, Mosby Elsevier, 2009.
10. **Fowler, M.E.,** Medicine and surgery of camelids, Ames, Iowa: Wiley-Blackwell, 2010.
11. **Heller, R.G.,** Pattern of motility and flow of digesta in the forestomach of the Llama (Lama guanacoe f. glama), Journal of Comparative Physiology B: Biochemical, Systemic & Environmental Physiology, 1984, 154, 529-533.
12. **Hoffman, E.,** The Complete alpaca book, Bonny Doon Press, Santa Cruz, California, 2006.
13. **Krautwald-Junghanns, M.E., Pees, M., Reese, S., Tully, T.,** Diagnostic Imaging of Exotic Pets, Schlutersche, 2010.
14. **Lechner-Doll, M.V.,** Particularities in forestomach anatomy, physiology and biochemistry of camelids compared to ruminants, Options Méditerranéennes: Série B, Etudes et Recherches, 1995, 13, 19-32.
15. **Smith, B.B.,** Third compartment ulcers in the llama, Veterinary Clinics of North America: Food Animal Practice, 1994, 10, 319-330.

16. **Tharwat, M.,** Ultrasonography of the abdomen in healthy and diseased camels (Camelus dromedaries), Journal of Applied Animal Research, 2020, 48, 300-312.
17. **Vallenas, A.C.,** A gross study of the compartmentalized stomach of two new-world camelids, the llama and guanaco, Journal of Morphology, 1971, 134, 399-423.
18. **Vater, A., Maierl, J.,** Adaptive Anatomical Specialization of the Intestines of Alpacas Taking into Account their Original Habitat and Feeding Behaviour, Anatomical Record (Hoboken), 2018, 301, 11, 1840-1851.
19. **von Engelhardt, W.A.,** Absorption and secretion in the tubiform forestomach (compartment 3) of the llama, Journal of Comparative Physiology, 1979, 132, 337-341.
20. **Vater, A.L., Zandt, E., Maierl, J.,** The topographic and systematic anatomy of the alpaca stomach, Anatomical Record, 2021, 304, 9, 1829-2067.

STUDY REGARDING THE CLINICAL-THERAPEUTIC MANAGEMENT OF RESPIRATORY DISEASE IN 9 CALVES

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Summary

The aim of the present paper is formulate out an integrated study of the results of our own investigations with the current ones from the specialized literature in the complex of respiratory diseases in cattle, mainly of the data regarding the clinical diagnostic algorithm and the optimization of their therapeutic approach. The first objective of the conducted studies was to identify the epidemiological and the inducing context, in order to optimize the clinical-therapeutic management of this condition in calves. The study was carried out in the period 2021 (January) - 2022 (March), within the Bucharest Sanitary-Veterinary Circumscription and the Gruiu farm, during which the disease was recorded in a number of 9 calves. The periodic clinical screening carried out at the level of this farm and the holdings of the Bucharest Veterinary Sanitary District, followed by early identification during the study period, allowed the structuring of patients thus affected into several groups, each benefiting from an individually modulated and adjusted antibacterial therapy in dynamics, depending on the therapeutic response and the evolution of the cases. In this sense, we tried to optimize the therapeutic approach, especially the anti-infective one, based on a curative efficiency. The groups of animals (lots) included patients classified on the basis of the intensity and extension of the pathological process, of clinical suspicion at the time of the preliminary evaluation. The criterion on the basis of which the study groups were created was based on the intensity and persistence of the functional and physical signs at the level of the respiratory system, the reduction or remission of which allowed the assessment of the degree of efficiency of the therapeutic protocol.

Keywords: calves, respiratory disease, therapy.

In intensive and semi-intensive breeding of bulls, diseases of the calves, especially respiratory and digestive diseases are responsible for numerous economic losses for farmers, both through morbidity and reduction of productive indices (2, 7, 20).

The immunological background, is not always prepared for the challenges related to the first months of life, makes young animals vulnerable to biotic aggressors, which in association with technological deficiencies and those related to environmental conditions in shelters and those related to nutrition, develop associated and known diseases under the name "respiratory disease complex", often a challenge for the veterinary practitioner and a financial strain for animal breeders (6, 10, 11, 17).

Materials and methods

The study in this paper was carried out during the period January 2021-March 2022 within the non-professional holdings of the Bucharest Sanitary-Veterinary District and Gruiu farm. The animals present are used for the commercial breeding system or in the domestic system, for own consumption.

The research was focused on a batch of 9 animals with various respiratory diseases, in different stages of evolution or produced by heterogeneous infectious agents. This group was fragmented into 3 groups of 3 animals each, for each group using a different protocol for therapeutic management.

In the present study, the cattle show symptoms suggestive of the complex of respiratory diseases in cattle, consisting of febrile syndrome, inappetence, productive cough and jetage.

Table 1

Study group of calves showing respiratory conditions

	NUMBER OF CALVES (n=9)			
BREED		**AGE**		**SEX**
		1-5 years	**6-10 years**	**M/F**
Limousine	***(n=4)***	***(n=4)***		**F=4**
Angus	***(n=2)***	***(n=2)***		**M=1** **F=1**
Bălțată românească	***(n=2)***	***(n=2)***		**F=2**
Metis	***(n=1)***	***(n=1)***		**M=1**

The applicability of general examination methods is necessary for a correct examination of patients, in order to establish a diagnosis and implicitly for the application of appropriate curative therapy. Thus, the approach in these cases was carried out according to the following protocol: anamnesis, clinical examination, therapeutic management. The anamnesis represents the first step in the clinical evaluation of the patient by providing valuable data regarding the patient's antecedents, either disease precursors or medical history (4, 5). Obtaining this information can determine the prognosis, orient the diagnosis or help to develop appropriate therapeutic attitudes. This provides important details on maintenance status, feed quality and housing conditions, data on the epidemiology of the herd or the patient itself (1, 3, 7, 11).

Fig. 1. Enroxil 5% (21)

Fig. 2. Draxxin (original photo)

The clinical examination is carried out by inspection, palpation, percussion and auscultation, that aims to collect data of clinical value, both direct and indirect, from a distance and close to the animal, in order to observe various pathological aspects that can lead to a medical finding, thus in following this method, the specific symptoms represented by: direct signs: wheezing, coughing, respiratory movements and indirect signs: pulmonary conditions (15, 18, 19).

Therapeutic management is used to improve and treat the symptoms, in an attempt to obtain the most effective results for the conditions described. As a first intention we used medication from two distinct pharmacological groups. A first category is represented by Enrofloxacin (Enroxil 5%) (Fig. 1), in a dose of 2.5 mg/kg body weight, subcutaneously or intravenously, with repetition every 24 hours, with a waiting time for meat of 10 days (9, 12, 13). The second category used in the treatment of conditions is Tulathromycin (Draxxin) (Fig.2), administered in a dose of 2.5 mg/kg, in a single dose, subcutaneously, with a resting time in the meat of 22 days (8, 14, 15).

Results and discussions

The Fig. 3 suggest the predisposition of females 77.7% (n=7) regarding the complex of respiratory diseases in young cattle, while males occupy a percentage of only 22.3% (n=2).

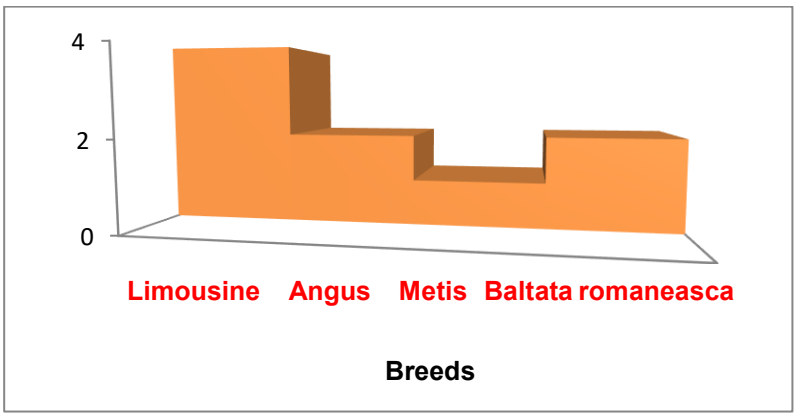

Fig. 3. Graphic representation of the gender of the patients from the study

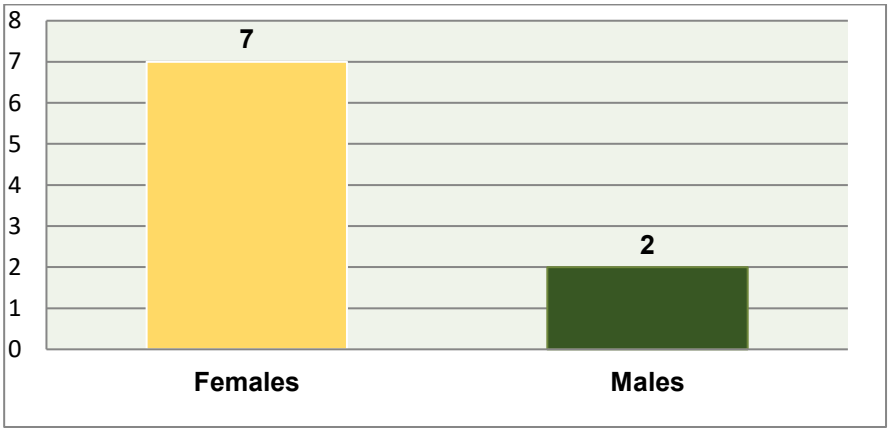

Fig. 4. Graphic representation of the races of the patients included in the present study

The above graphic (Fig. 4) shows the prevalence of the Limousine breed in our study, it occupies 44.4%, in second place are the Angus and Romanian Balțata breeds, each with a percentage of 22.2%, and in last place the Metis breed with a single case (11.1%).

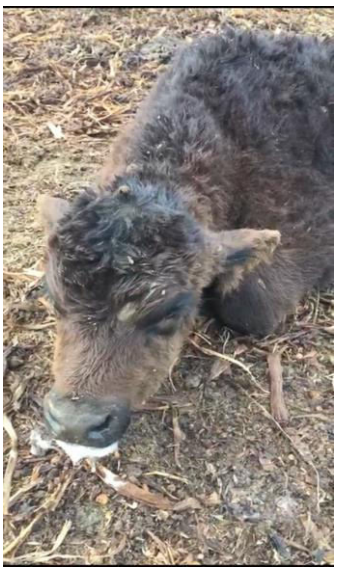

Fig. 5. Crossbreed calf diagnosed with lobular bronchopneumonia

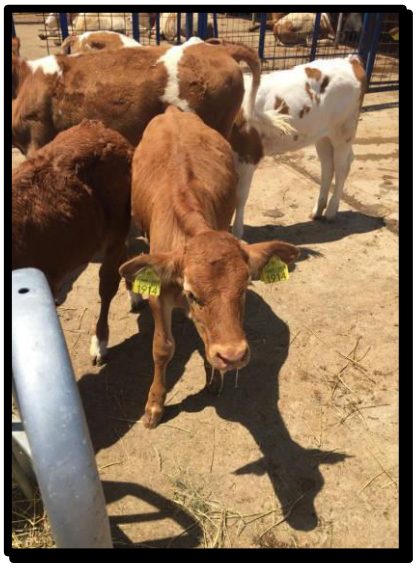

Fig. 6. Limousine breed calf diagnosed with macrobronchitis

Fig. 7. Bălțată Românească breed calf diagnosed with macrobronchitis

Fig. 8. Angus breed calf diagnosed with catarrhal bronchopneumonia

The symptomatological screening (Fig. 9) in young cattle diagnosed with complex respiratory diseases, was represented by febrile syndrome in 9 individuals, anorexia in 2 of them, sero-mucous discharge (n=5) and productive cough (n= 3).

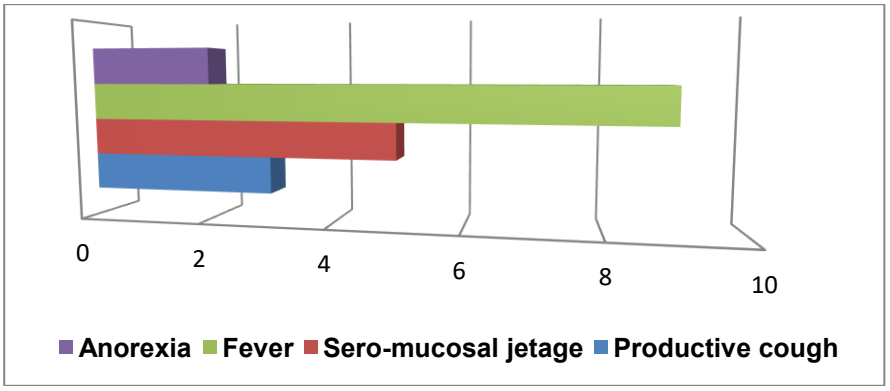

Fig. 9. Graphic representation of the predominant clinical signs in the study

The statistical analysis (Fig. 10) indicates that the largest share of the total number of cases is represented by the pathology of catarrhal bronchopneumonia (Fig. 8) with 34% percentage (n=3), being immediately followed by macrobronchitis (Fig. 6, 7) and lobular bronchopneumonia (Fig. 5), both in a percentage of 22%, the remaining two cases being diagnosed with crupal pneumonia and pulmonary congestion, each with a percentage of 11%.

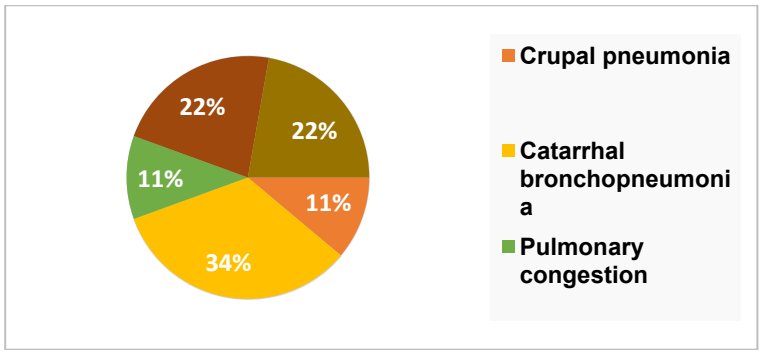

Fig. 10. Graphic representation of the clinical diagnoses of cases in the present study

This group was fragmented into 3 groups of 3 animals each, batch 1: received Enrofloxacin, batch 2: Tulathromycin and batch 3: received an associated treatment, Enrofloxacin and Tulathromycin. Animals in the third group, which received therapy for the management of respiratory manifestations with fluoroquinolones and macrolides, respectively Enrofloxacin and Tulathromycin, recorded the shortest time of remission of clinical symptoms, thus highlighting that this approach is the most effective way to improve symptomatology. Therefore, the anti-infective therapy with Enrofloxacin (sample no. 1) and Tulathromycin (sample no. 2) was effective, but it took a long time until the complete remission of the specific clinical signs (Fig. 11).

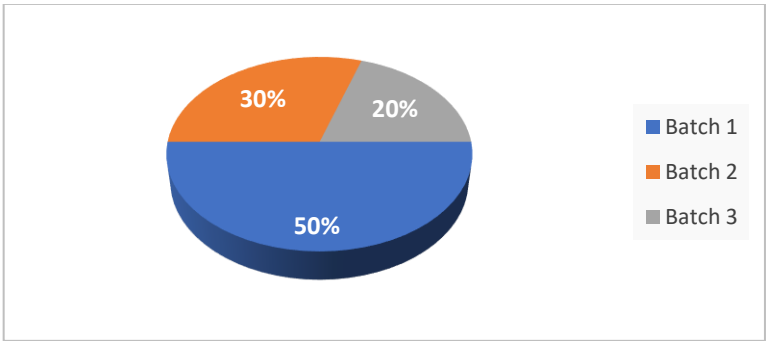

Fig. 11. Graphical representation of the average time in which the clinical signs were resolved according to the therapeutic management used for each sample

Conclusions

The low incidence of the complex of respiratory diseases in young bulls under medical supervision recognized a very low prevalence within the herds, much lower compared to the data provided by the specialized literature (8-14%) for this species, a situation attributable to the improvement of the exploitation systems and growth, where preventive measures are prioritized.

From the total number of patients included in our study (n=9), a higher percentage (77.7%) was recorded in females, which may indicate their predisposition to the occurrence of this complex of respiratory diseases, compared to males, raised and exploited under similar feeding and environmental conditions (with similar herd dysmicrobism).

In the patients in whom we used Enrofloxacin (2.5 mg/kg/day) as an anti-infective treatment, the reduction of the clinical expression of the fever syndrome and functional disorders was recorded after the first 3-4 administrations, and in the case of using the preparation Draxxin (tulathromycin in single dose of 2.5 mg/kg) the improvement of sympomatology occurred after 2-3 days.

The clinical-therapeutic management based on the use of antibacterial substances, namely Enrofloxacin and Tulathromycin - in batch 3, the fastest recovery (improvement of symptoms) was recorded, highlighting a superior curative efficiency of this combination, compared to the other two treatments used with the two active substances separately, in which the clinical remission was obtained much more difficult (3-5 days), by modulating and readjusting the dynamics of the treatment.

References

1. **Apley, M.,** The clinical syndrome of BRD: what it is and what it is not, Animal Health Research Reviews., 2014, 15, 2, 135-7.
2. **Blakebrough-Hall, C., Dona, A., D'occhio, M.J., McMeniman, J., González, L.A.,** Diagnosis of Bovine Respiratory Disease in feedlot cattle using blood H NMR metabolomics, Scientific Reports, 2020, 10, 1, 115.
3. **Borchers, A.T., Chang, C., Gershwin, M.E., Gershwin, L.J.,** Respiratory syncytial virus-a comprehensive review, Clinical Reviews in Allergy & Immunology, 2013, 45, 3, 331-79.
4. **Campbell, J.,** Bacterial Pneumonia in Cattle with Bovine Respiratory Disease Complex, MSD Manual, 2015.
5. **Campbell, J.,** Viral Infections Associated with Bovine Respiratory Disease Complex in Cattle, MSD Manual, 2015.
6. **Codreanu, I.**, Animal physiology, Printech, București, 2020.
7. **Codreanu, M.D.,** Medicina internă a animalelor domestice, Printech, București, 2020.
8. **Codreanu, M.D.,** Terapeutică veterinară, Printech, București, 2020.

9. **Crosby, S., Credille, B., Giguère, S., Berghaus, R.,** Comparative efficacy of enrofloxacin to that of tulathromycin for the control of bovine respiratory disease and prevalence of antimicrobial resistance in *Mannheimia haemolytica* in calves at high risk of developing bovine respiratory disease, Journal of Animal Science, 2018, 96, 4, 1259-1267.
10. **Deepak, Aly, S.S., Love, W.J., Blanchard, P.C., Crossley, B., Van Eenennaam, A.L., Lehenbauer T.W.,** Etiology and risk factors for bovine respiratory disease in pre-weaned calves on California dairies and calf ranches, Preventive Veterinary Medicine, 2021, 197, 105506.
11. **Ferraro, S., Fecteau, G., Dubuc, J., Francoz, D., Rousseau, M., Roy, J.P., Buczinski, S.,** Scoping review on clinical definition of bovine respiratory disease complex and related clinical signs in dairy cows, Journal of Dairy Science, 2021, 104, 6, 7095-7108.
12. **García-Galán, A., Seva, J., Gómez-Martín, Á., Ortega, J., Rodríguez, F., García-Muñoz, Á., De la Fe, C.,** Importance and Antimicrobial Resistance of *Mycoplasma bovis* in Clinical Respiratory Disease in Feedlot Calves, Animals (Basel), 2021, 11, 5, 1470.
13. **Guzman, E., Taylor, G.,** Immunology of bovine respiratory syncytial virus in calves, Molecular Immunology, 2015, 66, 1, 48-56.
14. **Jeffrey, L., Watts, Michael, T.S.,** Antimicrobial Resistance in Bovine Respiratory Disease Pathogens: Measures, Trends, and Impact on Efficacy, Veterinary Clinics of North America: Food Animal Practice, 2010, 26, 1, 79-88.
15. **Pardon, B., Buczinski, S.,** Bovine Respiratory Disease Diagnosis: What Progress Has Been Made in Infectious Diagnosis? Veterinary Clinics of North America: Food Animal Practice 2020, 36, 2, 425-444.
16. **Plumb, C.,** Plumb's Veterinary Drug Handbook: Pocket, 9th Edition, Wiley-Blackwell, 2018.
17. **Saif, L.J.,** Bovine respiratory coronavirus, Veterinary Clinics of North America: Food Animal Practice, 2010, 26, 2, 349-64.
18. **Vlăgioiu, C.,** Semiologie veterinară și tehnici de examinare, Sitech, Craiova, 2008.
19. **Zachary, F.J.,** Pathologic Basis of Veterinary Disease Expert Consult, 6th Edition, Elsevier, 2016.
20. ***https://www.beefresearch.ca/research-topic.cfm/bovine-respiratory-disease-38
21. *** https://shop.biotur.ro/

STUDY REGARDING THE THERAPEUTIC PROTOCOL IN CANINE PATIENTS WITH ACUTE GASTROINTESTINAL DISORDERS

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Summary

The aim of our case study is to identify an efficient treatment protocol for canine patients with gastrointestinal disorders, emphasizing on restoration of the hydro electrolytic balance and dietary changes. The prevalence of gastrointestinal disorders in dogs has increased constantly in the last years, being, most often, caused by errors in the patient's alimentation. The present study was conducted on 10 canine patients with various gastrointestinal disorders, to which a diagnosis protocol consisting of anamnestic data and paraclinical exams (blood test and ultrasonography) was applied. After establishing the diagnosis, a treatment protocol consisting of electrolytes, vitamin B supplements, psyllium, prebiotics, and diet changes was applied. Of the total number of patients (n=10), 70% (n=7) showed significant improvement of the clinical status just two days after starting the treatment protocol, with remission of the initial clinical signs (diarrhea, vomiting, apathy) and 30% (n=3) of the patients showed improvement 4-5 days after starting the treatment. The success rate of the treatment protocol was 100%, all patients' clinical status improved, the total remission of the clinical signs being observed in all cases in maximum 7 days after the onset of our therapeutic protocol.

Keywords: acute gastrointestinal disorders, canine patients, therapeutic protocol.

The role of nutrition in both acute and chronic gastrointestinal diseases is a current topic in veterinary medicine, and, at the same time, it has a major importance in the therapeutic management of patients diagnosed with pathologies from this area (1, 8, 9). A proper diet is essential for the prevention of nutrient deficiencies and malnutrition, repair of damaged intestinal epithelium, restoration of normal luminal bacterial populations, promotion of normal intestinal motility, and maintenance of normal immune functions. The amount of food ingested, the frequency of feeding, and the composition of the diet have important effects on the normal function of the gastrointestinal and may be used to help ameliorate signs of various digestive pathologies (17, 18, 20).

The present study was conducted on a group of canine patients with various gastrointestinal disorders, to which a diagnosis protocol consisting of anamnestic data and paraclinical exams (blood test and ultrasonography) was applied. After establishing the diagnosis, a treatment protocol consisting of electrolytes, vitamin B supplements, psyllium, prebiotics, and diet changes was applied. The results of our study highlight the importance of a well-balanced nutrition in both prevention and treatment of gastrointestinal disorders in dogs.

Materials and methods

The study was conducted on a group of 10 canine patients, between 1 and 9 years old, with clinical signs of acute gastrointestinal disorders, in the time period 2020-2021 at Medi Vet Constanța and Mel Vet Corbeanca veterinary clinics. The paraclinical exams performed included hematological investigations (complete blood count, liver transaminases, total protein) as an important diagnosis tool in carnivores (2, 4, 19) and ultrasound examination, ultrasonography being considered, according to the specialty literature, the golden standard for the diagnosis of gastro-intestinal disorders in carnivores (3, 5, 6, 12). After establishing the diagnosis, the treatment protocol based on nutritional changes was applied to all patients.

Results and discussions

All patients presented similar symptomatology features following the sudden change in diet: apathy, absent appetite, present hydric appetite, repeated diarrheal stools, normothermia, no painful sensitivity to abdominal palpation.

The details of the clinical exam in all 10 patients are presented in the synthetic Table 1.

Table 1

The main results regarding the anamnesis, the clinical exam, the time until the symptoms remission occurred and the time elapsed until full recovery, in the studied group of patients

No.	PATIENT'S NAME	BREED	AGE	SEX	RECTAL TEMPERATURE	EMESIS OCCURRENCE	FECES CONSISTENCY	ANAMNESIS	SYMPTOMS REMISSION	FULL RECOVERY
1	MAYA	MALTESE BICHON	2	F	38.0 °C (normothermia)	0	Low consistency	The patient ingested cat food	Two days after we started the therapeutic protocol	5 DAYS
2	LUNA	COCKER SPANIEL	5	F	39.2 °C (normothermia)	1	Low consistency	The patient received a large number of treats	Two days after we started the therapeutic protocol	5 DAYS
3	JACK	FRENCH BULLDOG	3	M	38.9 °C (normothermia)	1	Medium consistency	The patient's alimentation was changed suddenly	Two days after we started the therapeutic protocol	6 DAYS
4	LOULOU	AKITA INU	2	F	38.2 °C (normothermia)	0	Very low (watery) consistency	The patient received cow milk	Two days after we started the therapeutic protocol	5 DAYS
5	CLARA	MALTESE BICHON	4	F	39.1 °C (normothermia)	0	Low consistency	The patient receives constantly human food (ham)	Two days after we started the therapeutic protocol	4 DAYS
6	TOBY	POMERANIAN	8	M	38.8 °C (normothermia)	1	Normal consistency	The patient eats in the last 8 months cat wet food.	Four days after we started the therapeutic protocol	7 DAYS
7	SASHA	YORKSHIRE TERRIER	7	F	38.6 °C (normothermia)	0	Low consistency	The patient's alimentation was changed suddenly	Four days after we started the therapeutic protocol	7 DAYS
8	ZEUS	AMSTAFF	9	M	38.5 °C (normothermia)	0	Very low (watery) consistency	The patient has intolerance to the chicken meat protein	Five days after we started the therapeutic protocol	7 DAYS
9	SPOTT	MALTESE BICHON	5	M	38.4 °C (normothermia)	3	Normal consistency	The patient eats food scraps	Two days after we started the therapeutic protocol	5 DAYS
10	OTTY	BEAGLE	1	M	39.1 °C (normothermia)	0	Low consistency	The patient received human food (pizza)	Two days after we started the therapeutic protocol	5 DAYS

The most relevant ultrasonography images of the recovering patients are shown in Fig. 1.

The clinical picture of our patients was consistent with the data reported in the specialty literature regarding the specific symptomatology of the acute gastrointestinal disorders (4, 7, 14, 16).

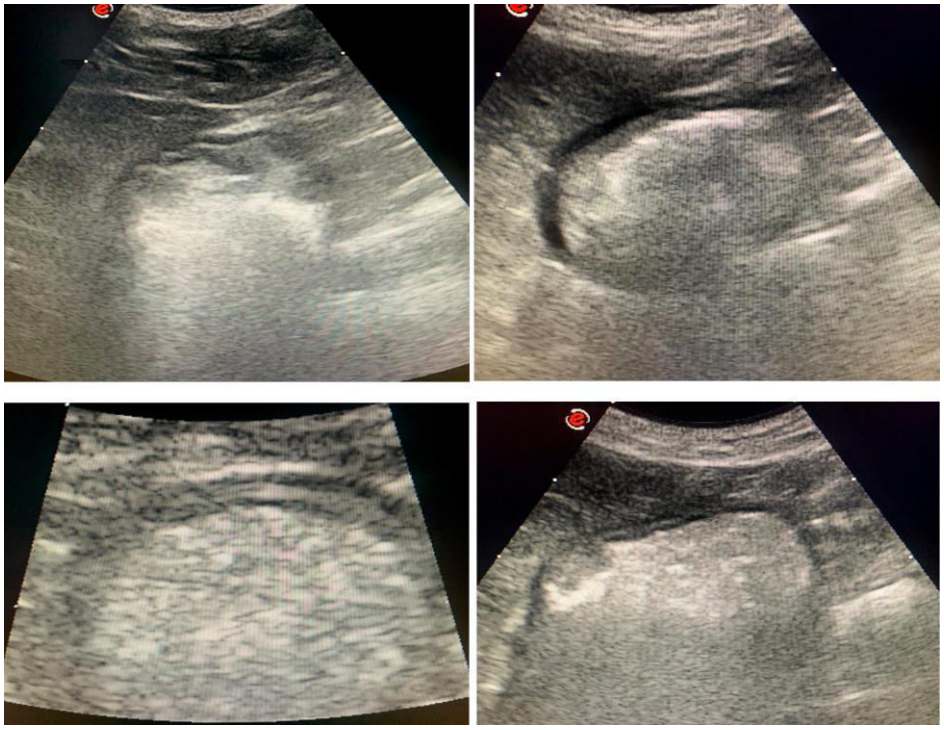

Fig. 1. Comparative ultrasonography before (up) and after (down) treatment, Maltese Bichon, F, 4 years old

After establishing the diagnosis, we applied the treatment protocol which mainly consisted of: electrolytes, vitamin B supplements, psyllium, prebiotics, and most important, diet change (gastrointestinal low fat special diet).

For each individual case, the ration was changed to an easily digestible, low-fat food that is balanced and rich in vital substances and that helps the patients assimilate the nutrients necessary for recovery. The food also contains fiber, prebiotics, psyllium seeds and a high content of electrolytes.

As a result of our treatment protocol, of the total number of patients (n=10), 70% (n=7) showed significant improvement of the clinical status two days after starting the treatment protocol, with remission of the initial clinical signs (diarrhea,

vomiting, apathy) and 30% (n=3) of the patients showed improvement 4-5 days after starting the treatment.

The results for each patient individually are presented in table 1 and the synthetic graphs (Fig. 2, Fig. 3).

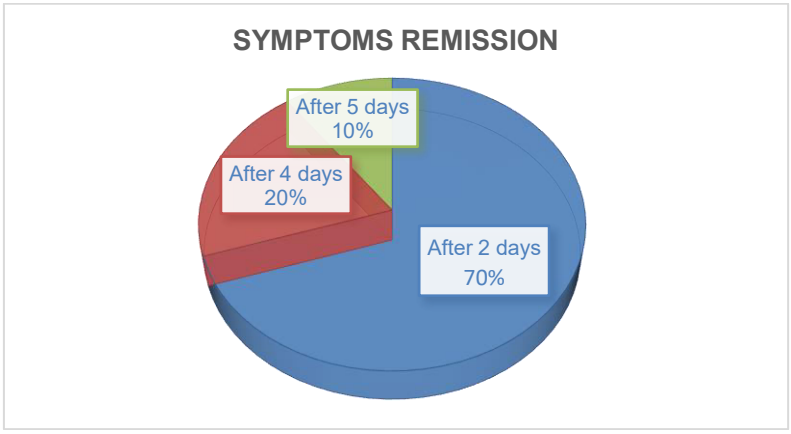

Fig. 2. Synthetic graph of the therapeutic protocol results – the symptoms remission in the group of studied patients

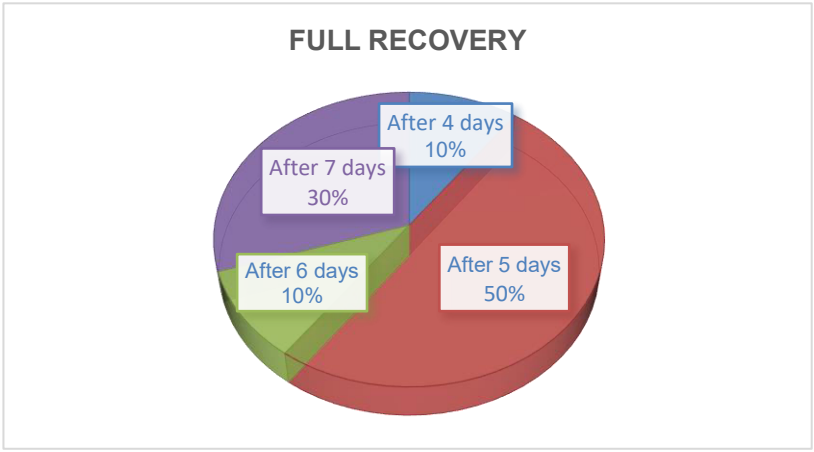

Fig. 3. Synthetic graph of the therapeutic protocol results – the full recovery in the group of studied patients

Also, as presented, 10% (n=1) of the patients showed full recovery after just 4 days of treatment, while half of the studied patients 50% showed full recovery after

5 days of treatment. The rest of the patients – 40% (n=4) showed full recovery after 6-7 days of treatment. Therefore, one week after the onset of the therapeutic protocol, 100% of the studied patients (n=10) showed full recovery.

The data we obtained is consistent with the results of other researchers regarding the impact of nutrition and applying a therapeutic protocol based on electrolytes, probiotics and dietary changes on canine patients of various ages and physiological conditions (10, 11, 13, 15).

Conclusions

The success rate of the therapeutic protocol we established and applied was 100%, all patients' clinical status improved, all patients showing symptoms remission 2-5 days after the onset of our therapeutic protocol, the total remission of the clinical signs being observed in all cases (100% of the studied patients) in maximum 7 days after the onset of our therapeutic protocol.

The study therefore highlights the importance of a well-balanced diet in the gastrointestinal disorders in dogs.

References

1. **Amorim, I., Taulescu, M.A., Day, M.J., Catoi, C., Reis, C.A., Carneiro, F., Gärtner, F.**, Canine Gastric Pathology: A Review, Journal of Comparative Pathology, 2016, 154, 1, 9-37.
2. **Codreanu, I., Uluitu, E., Codreanu, M.,** Investigations concerning hematological and biochemical profile in dog - german shepard breed, used for drug detection service. Simpozionul Ştiinţific Internaţional "35 ani de Învăţământ Superior Medical Veterinar în Republica Moldova", 15-16 octombrie 2009, Chişinău, 2009, 231-235.
3. **Codreanu, M.D., Popa, A. M.,** Repere ecografice în examinarea gastropatiilor la carnivorele de companie, Veterinaria An VII, 25/2017.
4. **Codreanu, M.D.,** Coordonate în abordarea clinico-terapeutică a maldigestiei-malabsorbţiei la câine, Veterinaria, 2013, 10, 20-27.
5. **Codreanu, M.D., Fernoagă, C., Cornilă, M., Codreanu, I., Crînganu, D., Turcitu, M.,** Correlation between the clinical and evolutive expression and type of ultrasound changes in the diagnosis of cavitary organs' diseases in dog. Lucrări Ştiinţifice - Medicină Veterinară, Universitatea de Ştiinţe Agricole şi Medicină Veterinară "Ion Ionescu de la Brad" Iaşi, 2010, 53, 3, 612-618.
6. **Dana, C., Codreanu, M.D., Moise, L., Florea, A.F.,** Ultrasound diagnosis in different gastrointestinal malfunctions in dogs. Lucrări Ştiinţifice - Universitatea de Ştiinţe Agronomice şi Medicină Veterinară Bucureşti. Seria C, Medicină Veterinară, 2005, 40, 281-284.
7. **Dunowska, M.,** What is causing acute haemorrhagic diarrhoea syndrome in dogs?, Veterinary Records, 2017, 180, 22, 539-541.

8. **Erdmann, C., Heilmann, R.M.,** Chronisch-entzündliche Darmerkrankungen beim Hund – diagnostische und therapeutische Aspekte [Diagnostic and therapeutic approach to chronic inflammatory enteropathies in dogs], Tierarztl Prax Ausg K Kleintiere Heimtiere, 2017, 45, 5, 317-327.
9. **Gazzola, K.M., Nelson, L.L.,** The relationship between gastrointestinal motility and gastric dilatation-volvulus in dogs, Topics in Companion Animal Medicine, 2014, 29, 3, 64-66.
10. **Jensen, A.P., Bjørnvad, C.R.,** Clinical effect of probiotics in prevention or treatment of gastrointestinal disease in dogs: A systematic review, Journal of Veterinary Internal Medicine, 2019, 33, 5, 1849-1864.
11. **Lenox, C.E.,** Nutritional Management for Dogs and Cats with Gastrointestinal Diseases, Veterinary Clinics of North America: Small Animal Practice, 2021, 51, 3, 669-684.
12. **Popa, A.M., Decei, L., Codreanu, I., Dutulescu A.V., Codreanu, M.D.,** Ultrasonographic appearances in canine intestinal lymphangiectasia, Journal of Biotechnology, 2018, 280, S75.
13. **Schmitz, S.S.,** Value of Probiotics in Canine and Feline Gastroenterology, Veterinary Clinics of North America: Small Animal Practice, 2021, 51, 1, 171-217.
14. **Stanton, M.E., Bright, R.M.,** Gastroduodenal ulceration in dogs. Retrospective study of 43 cases and literature review, Journal of Veterinary Internal Medicine, 1989, 3, 4, 238-44.
15. **Tolbert, M.K., Murphy, M., Gaylord, L., Witzel-Rollins, A.,** Dietary management of chronic enteropathy in dogs, Journal of Small Animal Practice, 2022, 63, 6, 425-434.
16. **Weber, M.P., Stambouli, F., Martin, L.J., Dumon, H.J., Biourge, V.C., Nguyen, P.G.,** Influence of age and body size on gastrointestinal transit time of radiopaque markers in healthy dogs, American Journal of Veterinary Research, 2002, 63, 5, 677-82.
17. **Wingate, D., Pearce, E., Ling, A., Boucher, B., Thompson, H., Hutton, M.,** Quantitative effect of oral feeding on gastrointestinal myoelectric activity in the conscious dog, Digestive Diseases and Sciences, 1979, 24, 6, 417-423.
18. **Zentek, J., Meyer, H.,** Normal handling of diets--are all dogs created equal?, Journal of Small Animal Practice, 1995, 36, 8, 354-9.
19. **Ziese, A.L., Suchodolski, J.S.,** Impact of Changes in Gastrointestinal Microbiota in Canine and Feline Digestive Diseases, Veterinary Clinics of North America: Small Animal Practice, 2021, 51, 1, 155-169.
20. **Zoran, D.,** Nutritional management of gastrointestinal disease, Clinical Techniques in Small Animal Practice, 2003, 18, 4, 211-217.

RESEARCH ON THE LEVEL OF SOME CELLULAR AND MOLECULAR EFFECTORS IN FALLOW DEER (*DAMA DAMA*)

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Summary

Due to favorable environmental conditions, in Romania live numerous species of wild game animals, specific to each region and vegetation. The fallow deer (*Dama dama*), along with other cervids, but also rabbit, wild boar and pheasant, represent the game species of major importance in our country, hunted for their meat, which possesses indisputable dietary qualities. The aim was to highlight the main haematological parameters in males (bucks) and females (does) of *Dama dama* species from three hunting grounds, in comparison to haematological parameters found in blood samples taken from ten young bulls at around two years old (stirks). For this, 30 blood samples of fallow deer were examined, twenty from bucks and ten from does, collected immediately after the animals were shot, while the control group consisted of 10 samples taken from the jugular vein of stirks. The results showed close average values in fallow deer with no significant differences between males and females. Monocytes and leukocytes were found to be significantly higher in wild ruminants (fallow deer bucks and does) than in domestic ruminants (stirks). In the fallow deer, a higher number of leukocytes was associated with a lower number of lymphocytes, while in stirks, the ratio was reversed.

Keywords: fallow deer, haematological parameters, immunity.

In our country, due to favorable environmental conditions, live numerous species of wild game animals, classified according to the vegetation zones, region and environments in which they reside. For continued development of the national hunting sector, it is necessary to know as much as possible about the biology of the species of hunting interest, so that by acting on their reproduction and distribution through modern methods of animal growth, husbandry and harvesting, the country's natural wealth can be used to its best advantage (4, 9, 19, 20).

According to official data, the fallow deer is numerically well represented on our country's western territory, mentioning that repopulations were also made in 27 other counties. For example, on the hunting grounds of Socodor, Arad county, there are over 1000 specimens, constituting the fallow deer reservation with the largest population of free-roaming deer in Europe. Also, on the territory of Luncoiul de Jos, Hunedoara county, there are 45 fallow deer spread across 600 hectares of land, constituting the Valea Lungă hunting reservation (2, 3).

Because of the special interest in the management and health status of breeding animals belonging to *Dama* genus (8), knowing the composition of the key haematological parameters is crucial for the identification and diagnosis of diseases and health issues affecting the deer population in the sylvatic environment, as well as from breeding farms (5, 6, 7, 9, 10, 15).

The aim of the current study was to highlight the main haematological parameters in males and females of *Dama dama* species from three hunting grounds in the counties of Arad and Timișoara, in comparison to the haematological parameters found in blood samples taken from ten young bulls at around two years old (stirks).

Materials and methods

To assess the main haematological parameters, 30 biological samples (blood) of fallow deer (*Dama dama*) were studied, twenty from males and ten from females, collected immediately after the animals were shot. The control group consisted of 10 samples taken from the jugular vein of two-year-old bulls.

The samples were collected in EDTA-treated vacutainers for the haematological, quantitative and qualitative analysis.

Quantitative haematological examination

The evaluation was carried out using the automated haematological analyzer MB-1830, which is suitable for both veterinary and human usage. The analyzer can determine the following haematological parameters: haematocrit (Ht); haemoglobin (Hb) concentration; mean corpuscular volume (MCV); mean corpuscular haemoglobin (MCH); mean corpuscular haemoglobin concentration (MCHC); red cell distribution width coefficient (RDW); platelet count and mean platelet volume (MPV).

Qualitative haematological examination

In order to determine the leukocyte formula, blood smears were made and examined with the microscope. The smears were created using the traditional approach, by spreading the drop of blood on a glass slide using a special, polished slide (16) and staining was done using two methods, respectively the May-Grünwald-Giemsa method in standard technique and the Diff-Quik method in standard technique (18). Smearing was done immediately after the animals were shot and solely with blood from fallow does and bucks. The smears were then observed using an Optika B-352 optical microscope and the leukocyte formula was determined by counting 200 cellular components and categorizing them (lymphocytes, monocytes, neutrophils, eosinophils and basophils).

Results and discussions

The total number of leukocytes and lymphocytes are substantially larger ($p \leq 0.05$) in fallow bucks compared to does, as can be seen in Table 1.

Table 1

Values of haematological parameters in fallow deer bucks and does, and stirks

Haematological parameters	Measuring unit	Fallow deer buck		Fallow deer doe		Stirks	
		X±SD	Limits	X±SD	Limits	X±SD	Limits
Erythrocytes	$x10^6/\mu l$	9.3±0.3	5.1-23.1	8.9±21.5	4.9-29.5	7.90±0.2	4.9 - 10
Hematocrit	%	**47.1±6.3**	35-54	45.2±6.4	34-49	**31.03±5.1**	22 - 33
Hemoglobin	g/100ml	16.8±1.5	9.8-20.6	16.3±2.1	8,2-21.1	12.98±1.3	8.5 -12
MCV	fl	52.2±7.1	46-68	52.8±6.8	42-669	43.94±6.4	38 - 50
MCH	pg	17.0±3.0	14-22	17.1±1	14-23	14.37±2.3	11 - 17
MCHC	g/dl	35.0±2.0	31-37	35.1±2	30-37	38.45±2.7	36 - 39
RDW	%	18.0±1.0	16-21	18.1±3	15-22	14.36±1.1	11 - 19
Leukocytes	$x10^3/\mu l$	**3.9±1.2**	1.3-6.5	**2.3±1.7**	1.0-5.7	**2.7±1.2**	1.8 - 5.3
Lymphocytes	$x10^3/\mu l$	**1.5±0.6**	0.8-4.3	**0.9±1.6**	0.6-2.7	**4.4±2.4**	4.9 - 12
Monocytes	$x10^3/\mu l$	0.5±0.1	0.1-0.4	**0.9±0.2**	0.1-0.5	**0.3±0.1**	0.1 - 0.5
Granulocytes	$x10^3/\mu l$	2.2±2.0	1.9-3.3	2.5±0.7	1.3-4.4	2,2±2.4	1.0 - 3.4
Platelets	$x10^5/\mu l$	4.4±2.1	3.2-7.3	4.1±2.6	3.2-7.2	4,7±2.3	4.0 - 6.6
MPV	fl	6.9±2.1	4.3-9.9	6.3±1.5	4.6-9.1	5.6±2.5	4.5 - 7.5

Legend: MCHC – mean corpuscular hemoglobin concentration; fl – femtoliter; MCH - mean corpuscular hemoglobin; MPV – mean platelet volume; RDW - red cell distribution width coefficient; MCV – mean corpuscular volume

Substantial differences in the number of leukocytes and the number of lymphocytes were identified, when comparing the evolution of the key haematological parameters in fallow deer to those in domestic ruminants (stirks).

Consequently, whereas the number of leukocytes is larger in fallow deer, the ratio was inverted in stirks, where the number of lymphocytes was much higher than the number of leukocytes. Also, the number of monocytes in fallow deer does was substantially greater than in stirks. The other analysed haematological parameters showed small differences based on the gender and species of the animals, but they were not statistically significant. Female fallow deer samples, on the other hand, showed a modest rise in the number of monocytes and a decrease in the number of granulocytes when compared to males.

The differences became apparent when the leukocyte formula was interpreted, when it was noted that stirks' proportion of lymphocytes is obviously larger than that of fallow deer men and females (Table 2, Fig. 1).

While analysing the leukocyte formula, was seen that calf lymphocyte counts were significantly greater than those of fallow deer males and females (Table 2, Fig. 1).

Table 2

Average values of leukocyte formula in fallow deer bucks

Crt. no.	Lymphocytes %	Neutrophils %	Eosinophils %	Basophils %	Monocytes %
1	26.47	52.36	1.89	0.70	18.85
2	35.59	43.50	2.25	0.56	18.07
3	30.74	48.50	2.21	0.60	17.95
4	25.99	53.36	2.10	0.50	18.05
5	34.59	48.50	2.25	0.56	18.07
6	25.16	**54.82**	1.95	0.27	17.80
7	29.25	50.50	2.05	0.50	17.70
8	26.20	52.26	1.99	0.70	18.85
9	33.49	45.58	2.10	0.76	18.07
10	31.47	47.57	1.80	0.80	18.36
X±SD (%)	**29.89±12.91%**	**49.69±7.25%**	**2.05±7.45%**	**0.59±26.12%**	**18.17±2.18%**
C.V.	**12.91%**	**7.25%**	**7.45%**	**26.12%**	**2.18%**

Legend: X = mean value; SD = standard deviation; CV = coefficient of variation

Observing the notable differences between the two blood parameters in wild and domestic ruminants, respectively the number of leukocytes and the number of lymphocytes, was noted that the individual variations were not very high, the maximum coefficient of variation (C.V.=24.7%) was found only in the case of the total number of lymphocytes of the fallow deer does (Table 3). The coefficient of variation for the other cell types ranged from 7.25% (neutrophils) to 26.12% (basophils) in fallow deer bucks, from 2.51% (monocytes) to 42.45% (basophils) in fallow deer does, and from 4.28% (lymphocytes) to 34.88% (eosinophils) in stirks.

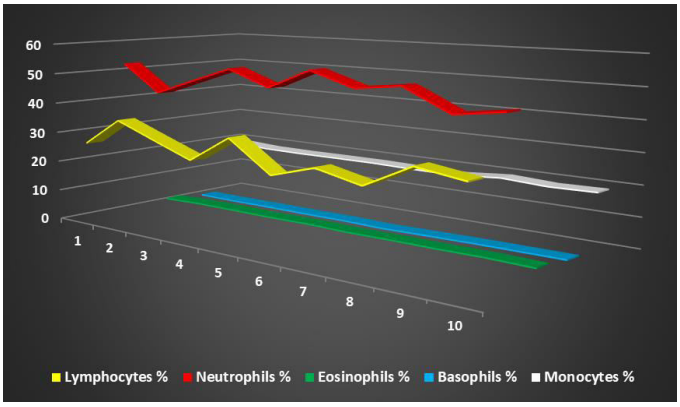

Fig. 1. The dynamics of leukocyte formula in the fallow deer bucks

The samples taken from fallow deer showed the greatest individual differences by cell category.

During the microscopic examination of the lymphocytes, the following morphological features were observed: the nucleo-cytoplasmic cells' ratio was in favor to the nucleus one, visible nucleoli in most cells and basophilic cytoplasm with many granules. This latter characteristic was more noticeable in fallow deer buck lymphocytes. The average values of the leukocyte formula in fallow deer females (Table 3, Fig. 2) were very comparable to those in bucks, with only minor deviations that were not statistically significant. Instead, substantial changes were detected when compared to stirks' findings (Table 4).

Table 3

Average values of leukocyte formula in fallow deer does

Crt. no.	Lymphocytes %	Neutrophils %	Eosinophils %	Basophils %	Monocytes %
1	27.49	50.45	1.80	0.90	19.36
2	21.26	58.82	1.47	0.07	18.38
3	23.19	56.50	1.69	0.77	17.85
4	25.66	53.36	2.15	0.66	18.17
5	29.93	49.50	2.01	0.61	17.95
6	42.16	37.80	1.70	0.59	17.75
7	28.73	50.55	2.05	0.50	18.17
8	27.20	52.86	1.75	0.29	17.90
9	43.77	35.58	2.00	0.55	18.10
10	31.44	47.87	1.69	0.75	18.25
X±SD (%)	**30.08± 24.7%**	**49.32± 15.07%**	**1.83± 11.59%**	**0.56± 42.45%**	**18.18± 2.51%**
C.V.	**24.7%**	**15.07%**	**11.59%**	**42.45%**	**2.51%**

Legend: X = mean value; SD = standard deviation; CV = coefficient of variation

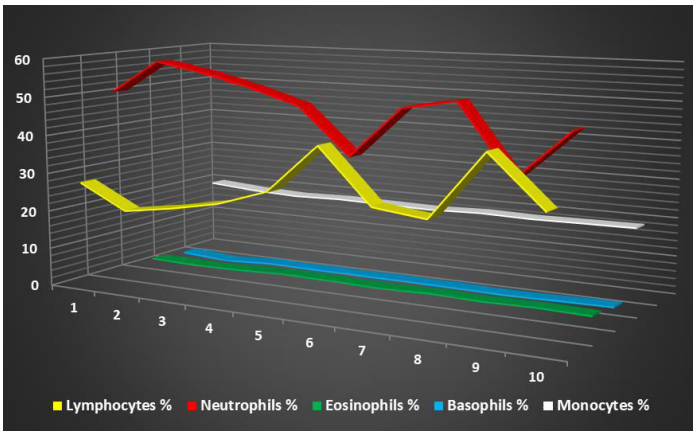

Fig. 2. Dynamics of the leukocyte formula in fallow deer does

Thus, the average values of monocytes obtained in samples collected from fallow deer females (18.18±2.51%), were much greater, compared to those discovered in stirks, where the minimum and maximum values were 2.94, respectively 5.19, with an average value of just 4.02±17.94% (Table 4, Fig. 3).

Significant differences in the lymphocyte-neutrophil ratio were discovered between the fallow deer and domestic bulls. The average percentage of lymphocytes in fallow deer does was 30.08±24.7%, whereas stirks had a value of 51.81±4.28%. On the contrary, the number of neutrophils was much larger in fallow deer does (49.32±15.07%) and bucks (49.69±7.25%), than in stirks, where the average value of neutrophils was smaller by almost 10%, correspondingly 40.53±4.34% (Table 4).

Table 4

Average values of leukocyte formula in stirks

Crt. no.	Lymphocytes %	Neutrophils %	Eosinophils %	Basophils %	Monocytes %
1	48.78	42.55	2.70	0.78	5.19
2	49.95	39.90	4.9	0.90	4.35
3	50.01	41.50	3.21	1.10	4.18
4	52.50	40.50	2.10	1.15	3.75
5	52.98	39.35	2.25	0.96	4.46
6	50.45	41.50	1.95	1.20	4.90
7	52.62	40.50	2.05	1.10	3.73
8	55.82	37.70	1.99	1.10	3.39
9	50.64	43.36	2.10	0.96	2.94
10	54.38	38.50	2.80	1.00	3.32
X±SD (%)	**51.81± 4.28%**	**40.53± 4.34%**	**2.60± 34.88%**	**1.02± 12.5%**	**4.02± 17.94%**
C.V.	**4.28%**	**4.34%**	**34.88%**	**12.5%**	**17.94%**

Legend: X = mean value; SD = standard deviation; CV = coefficient of variation;

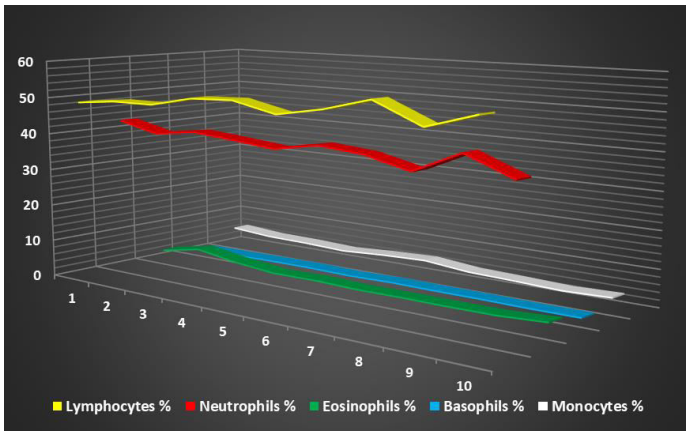

Fig. 3. Dynamics of leukocyte formula in stirks

When comparing the results from the two species, fallow deer (males and females) and stirks, were observed considerable variations between the two species in the main categories of cells engaged in both specific and non-specific immunity. For example, the proportion of monocytes in wild ruminants was substantially greater (18.18 ±2.51) than in domestic ruminants, where the average value was just 4.02 ±17.94. (Table 5).

Additionally, substantial differences in lymphocytes and neutrophils were discovered between the two species. However, it should be noted that between male and female fallow deer, the average values were much greater in females. Eosinophils and basophils were the only cell types whose values were relatively close across all three batches of animals.

Table 5

Percentage representation of white cells in fallow deer bucks, does and in stirks

Cell category	Bucks		Does		Stirks	
	X±SD (%)	Limits (%)	X±SD (%)	Limits (%)	X±SD (%)	Limits (%)
Lymphocytes %	29.89±12.91	19.5-35.0	**30.08±24.7**	20.5-38.0	**51.81±4.28**	30.5-65.0
Monocytes %	18.17±2.18	9.3-30.0	**18.18±2.51**	9.5-30.0	**4.02±17.94**	1.3-9.0
Neutrophils %	**49.69±7.25**	43.5-59.0	49.32±15.07	43.5-60.0	**40.53±4.34**	28.5-59.0
Eosinophils %	2.05±7.45	0,5-2.7	1.83±11.59	0,5-2.5	2.60±34.88	0,5-5.7
Basophils %	0.59±26.12	0.1-1.8	0.56±42.45	0.1-2.0	1.02±12.5	0.1-3.8

Legend: X = mean value; SD = standard deviation;

There are few publications in the specialized literature regarding the values of the main haematological parameters from wild ruminants, in general, and fallow deer (*Dama dama*), in particular. Nevertheless, Allen et al. evaluated various haematological parameters (erythrocytes (10^6/mm^3), hematocrit (%), hemoglobin (g/100 mL), leukocytes (10^3), neutrophils (%), lymphocytes (10^3), monocytes (10^3), eosinophils (10^3), basophils (10^3)), including the neutrophils:lymphocytes ratio. The scientists evaluated 176 animals classified in four age categories: 1-5 months, 6-11 months, 12-17 months and 18 months and older. The authors obtained, after laboratory examinations, the following average values of the monitored haematological parameters: erythrocytes 9.2 ± 0.2x10^6/mm^3, hematocrit 46.7 ± 0.6%, hemoglobin 16.4 ± 0.3 g/100 ml, mean concentration of erythrocyte hemoglobin 35.1 ± 0.2%, and total leukocytes 3.0 ± 0.1 10^3/mm^3 (1).

The authors stated that the values obtained for the main haematological parameters, namely the hematocrit, hemoglobin level and number of neutrophils, lymphocytes and basophils, were not changed by the evolution of the climatic circumstances of the corresponding year. Moreover, no significant differences were

discovered based on age, gender, or season. Instead, large variations in other cellular variables were mostly related with years in which considerable declines in yearly precipitation were registered. Thus, a considerable seasonal variation in the numbers of leukocytes and those of monocytes was discovered in deer over the age of 18 months (1).

Furthermore, the authors observe, after comparing the obtained data, that behavior, hunting background, altitude, physical condition, stage of gestation, stage of horn growth, or time of blood collection have no significant impact on the evolution of the main categories of cells (1).

However, these results are disproved by Poljičak-Milas et al. (2009), who researched some comparative haematological analyses carried out on blood samples collected from pregnant and non-pregnant red deer does (*Cervus elaphus*) and fallow deer does (*Dama dama*). They discovered in pregnant females belonging to both breeds, a significant decrease in the number of erythrocytes (RBC), hemoglobin concentration (Hb) and hematocrit level (Ht) compared to non-pregnant females. On the other hand, red deer was found to have higher hemoglobin and hematocrit concentrations than fallow deer does (11, 12).

Differences in mean erythrocyte volume (MCV) and erythrocyte hemoglobin (MCH) between females of the two species were also discovered in red blood cell values. Thus, MCV was shown to be lower in pregnant fallow deer females than in non-pregnant ones, but MCV was slightly higher in pregnant red deer than in non-pregnant ones. There were also differences in the number of leukocytes for non-pregnant *Cervus elaphus* females, which had a larger number of leukocytes than the pregnant ones. Also, non-pregnant fallow deer females had the lowest number of leukocytes among all categories of animals studied. The number of neutrophils and lymphocytes determined in females belonging to *Cervus elaphus* species was higher, compared to fallow deer does (11, 12).

The authors didn’t observe any significant differences in the number of neutrophils between pregnant and non-pregnant females, but a lower number of lymphocytes was found in pregnant *Cervus elaphus* deer than in non-pregnant females, while pregnant fallow deer does had a twice higher number of lymphocytes than non-pregnant females (11, 12).

According to some researchers, age, sex and season have no effect on hematocrit, hemoglobin level, the number of lymphocytes, segmented neutrophils, or basophils in mature or immature deer. Instead, significant variation in erythrocytes, mean erythrocyte volume, mean hemoglobin concentration, percentage of segmented neutrophils, percentage of lymphocytes and neutrophil to lymphocyte ratio can be correlated with age in mature deer (1, 13, 14, 17).

Conclusions

The studied haematological parameters showed close average values in fallow deer, both bucks and does, with no significant differences between males and females.

Among the evaluated haematological parameters, the number of leukocytes, lymphocytes and monocytes determined in the three categories of animals showed significant differences between wild and domestic ruminants.

Monocytes and leukocytes were found to be significantly higher in wild ruminants (fallow deer bucks and does) than in domestic ruminants (stirks).

In the fallow deer, a higher number of leukocytes was associated with a lower number of lymphocytes, while in stirks, the ratio was reversed.

References

1. **Allen, E., Medin, A.E., Bowden, D.C.,** Erythrocytes and Leukocytes in a Colorado Mule Deer Population, Journal of Wildlife Management, 1970, 34, 2, 389-406.
2. **Apollonio, M., Andersen, R., Putman, R.J.,** European ungulates and their management in the 21st century, Cambridge England: Cambridge University Press, 2010.
3. **Arslangündogdo, Z., Kasparek, M., Saribasak, H., Kacar, M.S., Yöntem, O., Sahin, M.T.,** Development of the population of the European fallow deer, *Dama dama dama* (Linnaeus, 1758) in Turkey, Zoology in The Middle East, 2010, 49, 3-12.
4. **Bucur, I., Dumitrescu, V., Imre, K., Herman, V., Nichita, I., Cristina, R.T., Tîrziu E.,** Research on the frequency of resistance phenotypes in bacterial strains isolated from chamois (*Rupicapra rupicapra carpatica*), Revista Română de Medicină Veterinară, 30, 1, 66-70.
5. **Chapman, D.I., Chapman, N.G., Kent, J.E.,** Some serum constituents of fallow deer (*Dama dama*) in England, Research in Veterinary Science, 1980, 29, 105-107.
6. **English, A.W., Lepherd, E.E.,** The haematology and serum biochemistry of wild fallow deer (*Dama dama*) in New South Wales, Journal of Wildlife Diseases, 1981, 17, 289-295.
7. **Esattore, B., Saggiomo, L., Sensi, M,·Francia, V., Cherin M.,** Tell me what you eat and I'll tell you where you live: an updated review of the worldwide distribution and foraging ecology of the fallow deer (*Dama dama*), Mammalian Biology, 2022, 102, 321-338.
8. **Forsyth, D.M., Thormson, C.L., Hartley, J., Mackanzie, D.I., Price, R., Wright, E.F., Mortimer, J.A.J., Nugent, G., Wilson, L., Livingstone, P.,** Long term changes in the relative abundances of introduced deer in New Zealand

estimated from faecal pellet frequencies, New Zealand Journal of Zoology, 2011, 38, 237-249.
9. **Howerth, E.W., Nemeth, N.M., Ryser-Degiorgis, M.P.,** Cervidae, In: Pathology of Wildlife and Zoo Animals, Academic Press London, 2018.
10. **Husband, A.J.,** Ungulate Immune Systems, Encyclopedia of Immunology, 1998, 2449-2451.
11. **Poljičak-Milas, N., Marenjak, T.S., Slavica, A., Janicki, Z., Filipović, N., Sruk, V.,** Comparative hematological and biochemical values in pregnant and non-pregnant red, *Cervus elaphus*, and fallow deer, *Dama dama*, females, Folia Zoologica, 2009, 58, 1, 36-44.
12. **Poljicak-Milas, N., Slavica, A., Janicki, Z., Robic, M., Belic, M., Milinkovic-Tur, S.,** Serum biochemical values in fallow deer (*Dama dama L*.) from different habitats in Croatia, European Journal of Wildlife Research, 2004, 50, 7-12.
13. **Ranucci, S., Morgante, M., Diverio, S., Costarelli, S., Beghelli, D., Duranti, E.,** Blood constituents and clinical findings in captured fallow deer (*Dama dama*), Supplemento alle ricerche di biologia della selvaggina, 1996, 25, 209-217.
14. **Rehbein, S., Bienoschek, S., Sachse, M., Neubert, E.,** Hämatologische und klinisch-chemische Untersuchungen bei natürlich und bei mutterlos aufgewachsenen Damhirschen (*Dama dama L*.) – 2, In: Klinisch-chemische Untersuchung im Blutplasma, Der Zoologische Garten, 1999, 69, 89-108.
15. **Reid, T.C., Towers, N.R.,** Blood parameters of normal farmed deer, Journal of the Royal Society of New Zealand, 1985, 22, 73-76.
16. **Şincai, M.,** Tehnici de citologie normală şi patologică, Ed. Mirton, Timişoara, 2000,
17. **Vengušt, G., Bidovec, A.,** Some serum chemistry values of fallow deer (*Dama dama L*.) in Slovenian hunting enclosures, Veterinarski Arhiv, 2002, 72, 4, 205-212.
18. ***Ellis, R.,** Diff-Quick (Diff-Quik) Staining Protocol, in IHC World Life Science Products & Services, 2012, www.ihcworld.com/_protocols/special_stains/diff_quick_ellis.html.
19. ***https://biblioteca.regielive.ro/proiecte/agronomie/evolutia-efectivelor-de-vanat-borsec-88787.html
20. ***https://silvanews.ro/silvicultura/cinegetica-salmonicultura/speciile-de-interes-vanatoresc-din-romania

SERUM LYSOZYME AND SERUM PROPERDIN IN FALLOW DEER (*DAMA DAMA*)

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Summary
Immunity is the capacity of each organism to create a proper immune response after being exposed to an antigen (infections or diseases produced by potentially pathogenic microorganisms). Mammals utilize both, innate and adaptive immune systems together to protect themselves against infectious and non-infectious stressors. As in domestic animals, the immunocompetence of wild animals is controlled by endogenous and exogenous factors. Moreover, the defensive capacity of the animals is also influenced by the amount of morphological and functional development of the humoral and cellular primary effectors that participate in the immune reactions. Through serological and immunological tests, sufficient data can be gathered for the characterization of the immunological profile and immunological mechanisms, in order to accurately assess the immune system's response capabilities. On this premise, the study aimed to examine non-specific immunity in wild ruminants (*Dama dama)* using two humoral immunological indicators, lysozyme and serum properdin, whose analysis provides solid data on an organism's immune response potential.
Keywords: lysozyme, fallow deer, immunity, serum properdin.

The immune system has continuously developed, along with phylogenetic evolution, being the body's defense system aimed at neutralizing all possible noxes that affect or may affect the homeostasis of the host. Practically, immunity represents the ability of each organism to produce a normal immune response, after exposure to an antigen (infections or diseases produced by potentially pathogenic microorganisms) (2, 3, 20).

Thus, mammals use innate and acquired immune mechanisms to cope with a multitude of infectious and non-infectious stressors. Innate and adaptive immune mechanisms are complementary and synergistic. Adaptive mechanisms (antibodies and antigen-specific T lymphocytes) are used whenever primary, non-specific mechanisms fail to control the homeostasis challenge and ruminants are no exception to this general rule (1, 2, 5, 8, 9, 12, 20).

Equivalently to domestic animals, the immunocompetence of wild animals is influenced by numerous factors, both endogenous and exogenous, among which we can mention food intake, environmental conditions, including season, stress, sex,

etc. There are opinions according to which immunocompetence shows an accentuated sexual dimorphism, respectively females show a general increased immunoreactivity compared to males (1, 2**,** 9, 11, 15, 18, 19).

The defense capacity of an organism is also conditioned by the level of morphological and functional development of the main effectors, humoral and cellular, that intervene in immune reactions. Deciphering immunological mechanisms, with the help of serological tests, allows obtaining objective values, which define what we call "immune status" or immunological profile. "Conventional" immunological tests provide sufficient data for a correct evaluation of the reaction capacity of the immune system (4, 6, 7, 13, 14).

On this basis, the research aimed to assess non-specific immunity in wild ruminants through two humoral immunological parameters, respectively lysozyme and serum properdin, whose investigation provides concrete data on the immune response capacity of an organism.

Materials and methods

To determine the main hematological parameters, 30 blood samples were analyzed, respectively ten samples obtained from males and ten samples from females of fallow deer (*Dama dama*), collected immediately after the animals were shot. The control group was represented by ten samples collected from two-year-old stirks from the jugular vein. The samples were collected in vacuum containers with EDTA for hematological, quantitative and qualitative examinations.

Serum lysozyme dosing

Serum lysozyme concentration was determined by the diffusimetric method, adopted from Pasteur Institute, Bucharest (17). On a Petri plate, in which a culture of *Micrococcus lysodeicticus* has been incorporated, were applied the serum samples to be analyzed in wells practiced in the gel. Through the diffusion of lysozyme from the serum, bacterial cell lysis takes place, forming, around the well, a halo whose diameter is proportional to the concentration of serum lysozyme that lyses the germs included in the medium.

The following formula was used to calculate the concentration of lysozyme contained in the investigated samples:

$$y = 91x + \frac{6}{x} + 4.5$$

where:

y = the lysis zone diameter;

x = the lysozyme concentration (mg/100 ml).

Serum properdin dosing

The concentration of serum properdin was determined using the colorimetric method, a method adopted from the Pasteur Institute, Bucharest, and the principle of the method consists in the spectrophotometer colorimetric determination of properdin isolated by complexation on inulin and treated with Biuret reagent (17).

The properdin concentration calculation, expressed in mg%, was made using the formula:

properdin mg% = (ODs-ODbs) x 2 x Correction factor

where: ODs = sample optical density;
ODbs = blank sample optical density;
Correction factor = 269 (constant).

Results and discussions

The non-specific humoral defense factors, serum lysozyme and serum properdin, are not modified in the case of animals from the control group, throughout the experimental period. Thus, the recorded values are within the physiological limits of the species and this allows their admission as reference values in estimating the immunomodulatory effect of the two substances (Table 1, 2 and Fig. 1, 2).

Serum lysozyme dynamics

Serum lysozyme showed very large differences, from one animal to another and from one category of ruminant (wild or domestic) to another, with the mention that the coefficient of variability was between 6.34 and 14.67% (Table 1, Fig. 1).

In fallow deer males, the serum lysozyme values were between 50.0 mg/100 cm^3 serum, a value recorded on a single sample, and 88.5 mg/100 cm^3 serum, with the mention that values above 80.0 mg/100 cm^3 serum or very close to this in seven of the ten animals studied. The serum lysozyme average value, in the batch of ten studied animals, was 75.75±10.54 mg/100 cm^3 serum, with a variability coefficient of 14.67%.

On the other hand, in does (females fallow deer), serum lysozyme values recorded significant differences compared to males, of approximately ten mg/100 cm^3, the average of the recorded values being 84.4±7.62 mg/100 cm^3, compared to 75.75±10.54 mg/100 cm^3 serum. On the contrary, relatively small differences were found between the ten does taken in the study, between 70.0 and 96.6 mg/100 cm^3 serum with a variability coefficient much lower than in bucks (males fallow deer), respectively of 9.6%.

Compared to wild ruminants, in domestic ruminants, respectively in the ten stirks, serum lysozyme values were much lower, the differences being highly significant. Thus, the differences between the average values of the two ruminant categories were 71.36 mg/100 cm^3 serum compared to does and 62.71 mg/100 cm^3

serum compared to bucks. In stirks, the mean value of serum lysozyme concentration was only 13.04 mg/100 cm^3 serum, with a minimum of 12.0 mg/100 cm^3 and a maximum of only 14.5 mg/100 cm^3 serum, and a coefficient of variation of 6.34% (Table 1).

Table 1

Serum lysozyme concentration in the studied animals

Sample	The measure unit	Buck (Male fallow deer)	Doe (Female fallow deer)	Stirk (1-2 y.o. bulls)
1	mg/100 cm^3 serum	86.0	96.6	13.4
2		77.5	78.0	12.5
3		81.5	88.5	14.5
4		50.0	70.0	12.0
5		65.5	78.0	14.0
6		72.5	84.5	13.0
7		77.5	92.0	12.5
8		88.5	88.6	12.0
9		78.5	89.4	13.5
10		80.0	78.4	13.0
Mean (X)		75.75	84.4	13.04
Standard Deviation (Sx)		10.54	7.69	0.78
Variability Coefficient (CV)%		14.67	9.60	6.34

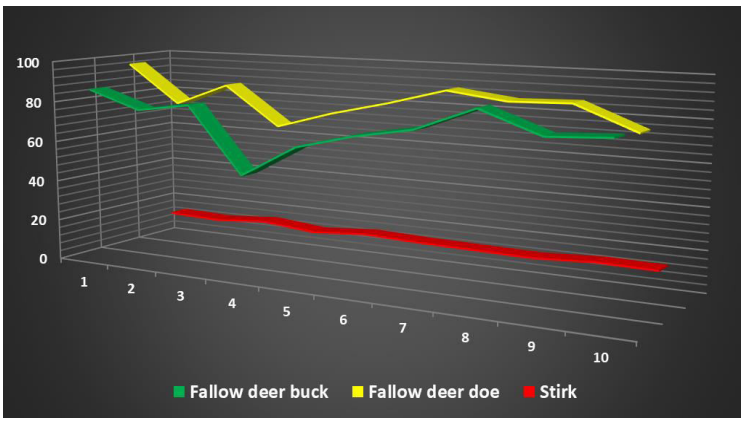

Fig. 1. Serum lysozyme dynamics

The results obtained by us in calves are quite close to those obtained by Tîrziu et al. and Jung et al., who, following some studies on immunity in these animals, found a dynamic of the serum lysozyme concentration, positively influenced by the administration of some immunogens. However, it can be stated that even in the case of immune stimulation, serum lysozyme values did not change significantly, compared to those found in wild ruminants (10, 16).

Thus, was observed that the average values of serum lysozyme, determined in wild ruminants were extremely significantly higher, compared to those found in domestic ruminants ($P \leq 0.01$), specifying that even a maximum value was recorded in does with 84.6 mg/100 cm^3 serum higher than the minimum value found in stirks, respectively 12.0 mg/100 cm^3 serum. Based on these results, it can be stated that non-specific immunity, in wild ruminants, has an essential role in maintaining general immunostasis.

Serum properdin dynamics

The properdin concentration had a similar evolution to that observed with lysozyme, with the mention that the average values of this parameter were significantly higher, compared to those of lysozyme, in all three categories of animals studied (Table 2 and Fig. 2). Thus, in fallow deer, the average serum properdin value was 95.41 µg/100 cm^3 serum, with limits between 80.0 (the minimum limit, recorded in one animal) and 104.5 µg/100 cm^3 serum (value recorded in two animals) and a variability coefficient of 8.03% (Table 2).

Table 2

Serum properdin concentration in the studied animals

Sample	The measure unit	Buck (Male fallow deer)	Doe (Female fallow deer)	Stirk (1-2 y.o. bulls)
1	µg/100 cm^3 serum	92.2	93.5	17.2
2		98.0	102.0	18.5
3		102.4	98.5	19.1
4		88.6	110.5	17.5
5		92.4	88.0	18.5
6		80.0	104.2	18.0
7		104.0	102.8	19.2
8		104.5	98.8	17.5
9		98.5	112.0	18.5
10		93.5	106.4	19.0
Mean (X)		95.41	101.67	18.3
Standard Deviation (Sx)		7.30	6.98	0.68
Variability Coefficient (CV)%		8.03	7.23	3.92

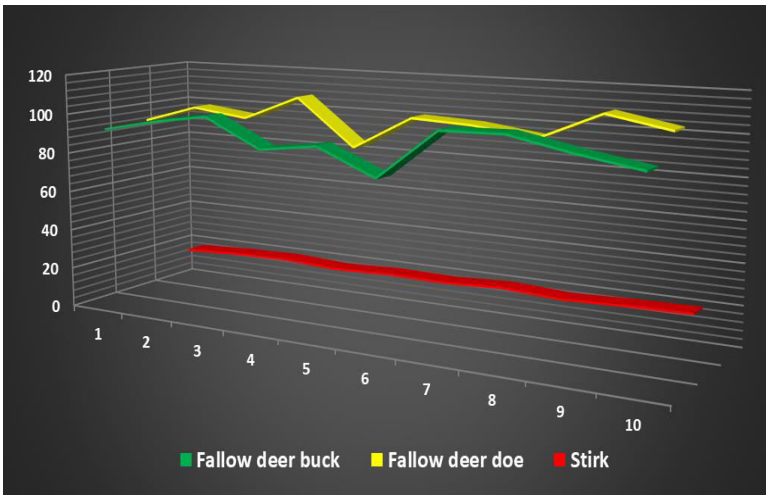

Fig. 2. Serum properdin dynamics

Analyzing the results obtained in the two categories of ruminants, domestic and wild, was observed that both serum lysozyme and serum properdin recorded much higher values in wild ruminants, compared to domestic ones, both individually and on arithmetic mean for the ten animals taken in the study (Table 3, Fig. 3).

Table 3

Mean values of lysozyme and properdin in the studied animals

Animal category	Serum lysozyme (µg/ml ser)	Serum properdin (mg/100ml ser)
Buck (Male Fallow Deer)	75.75±10.54* 14.67**	95.41±7.26* 8.03**
Doe (Female Fallow Deer)	84.40±7.68* 9.60**	101.67±6.98* 7.23**
Stirk (1-2 y.o. calves)	13.04±0.78* 6.34**	18.3±0.68* 3.92**

The legend: * - arithmetic mean ± standard deviation; ** - the variability coefficient.

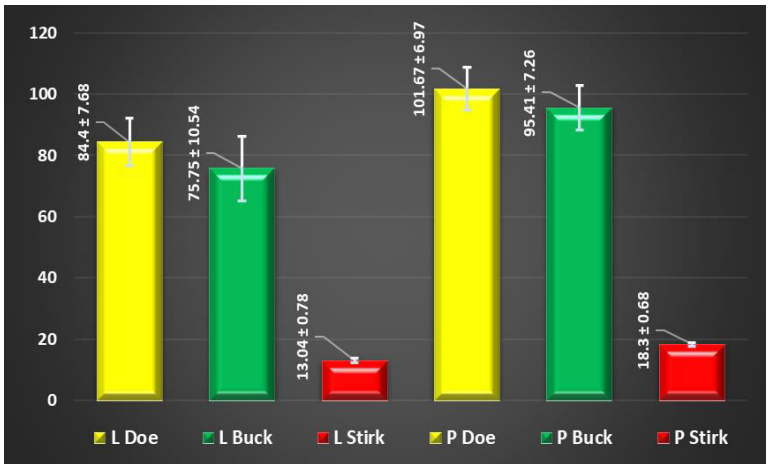

Fig. 3. Mean values of serum properdin (mg/100 ml serum) and serum lysozyme (µg/ml serum)

These values of non-specific humoral factors definitely influence the natural immunity in wild ruminants, which could explain the extremely low number of infectious diseases in these animals. Analyzing these values, was noticed that the recorded values were not influenced by age, hunting background, nutrition or other intrinsic and extrinsic factors, the only factor that influenced the evolution of these non-specific immune effectors, although to a small extent, being the sex of the animals.

In fallow deer, both serum lysozyme and serum properdin have higher values in females compared to males, with no significant differences, as can be seen from the results presented in the mentioned tables and graphics.

Conclusions

Among the non-specific humoral factors, serum lysozyme and serum properdin registered differences, between fallow deer males and females, the values being higher in females for both investigated parameters.

Compared to wild ruminants, in stirks for all studied animals, serum lysozyme and serum properdin recorded much lower values, even three, four times.

Based on the obtained results, it can be stated that in wild ruminants, compared to domestic ones, the level of non-specific humoral immune parameters reaches much higher values, which ensures an extremely effective non-specific immunity in case of contamination with the microorganisms with which they come into contact.

References

1. **Amadori, M., Stefanon, B., Sgorlon, S., Farinacci, M.,** Immune system response to stress factors, Italian Journal of Animal Science, 2009, 8, 287-299.
2. **Amadori, M.,** The Innate Immune Response to Noninfectious Stressors: Human and Animal Models, Academic Press, London, UK, 2016.
3. **Bronzo, V., Lopreiato, V., Riva, F., Amadori, M., Curone, G., Addis, M.F., Cremonesi, P., Moroni, P., Trevisi, E., Castiglioni, B.,** The Role of Innate Immune Response and Microbiome in Resilience of Dairy Cattle to Disease: The Mastitis Model, Animals (Basel), 2020, 10, 8, 1397.
4. **Chapman, D.I., Chapman, N.G., Kent, J.E.,** Some serum constituents of fallow deer (*Dama dama*) in England, Research in Veterinary Science, 1980, 29, 105-107.
5. **Dumitrescu, V., Borlea, F., Nichita, I., Bucur, I., M., Tîrziu, E.,** Comparative research on antimicrobial resistance in bacteria isolated from domestic and wild animals (chamois - *Rupicapra rupicapra*), Lucrări ştiinţifice – USAMVB „Regele Mihai I al României" Timișoara, Medicină Veterinară, LII, 1, 54-60.
6. **English, A.W., Lepherd, E.E.,** The haematology and serum biochemistry of wild fallow deer (Dama dama) in New South Wales, Journal of Wildlife Diseases, 1981, 17, 289-295.
7. **Giovannini, A., Canceflotti, F.M., Turilli, C., Randi, E.,** Serological investigations for some bacterial and viral pathogens in fallow deer (*Cervus dama*) and wild boar (*Sus scrofa*) of the san rossore preserve, Tuscany, Italy, Journal of Wildlife Diseases, 1988, 24, 1, 127-132.
8. **Haley, P.J.,** Species differences in the structure and function of the immune system, Toxicology, 2003,188, 49-71.
9. **Husband, A.J.,** Ungulate Immune Systems, Encyclopedia of Immunology, 1998, 2449-2451.
10. **Jung, M., Shin, M.K., Cha, S.B., Shin, Seung, W., Yoo, A., Lee, W.J., Park, H.T., Park, H.J., Kim, H.B., Jung, Y.K., Yoo, H.S.,** Supplementation of dietary germanium biotite enhances induction of the immune responses by foot-and-mouth disease vaccine in cattle, BMC Veterinary Research, 2014, 10, 179.
11. **Morse, B.W., Miller, D.L., Miller, K.V., Baldwin, C.A.,** Population health of fallow deer (*Dama dama*) on little St. Simons island, Georgia, USA, Journal of Wildlife Diseases, 2009, 45, 2, 411-421.
12. **Palmer, M.V., Whipple, D.L., Olsen, S.C., Jacobson, R.H.,** Cell mediated and humoral immune responses of white-tailed deer experimentally infected with *Mycobacterium bovis*, Research in Veterinary Science, 2000, 68, 95-98.
13. **Poljicak-Milas, N., Slavica, A., Janicki, Z., Robic, M., Belic, M., Milinkovic-Tur, S.,** Serum biochemical values in fallow deer (*Dama dama L.*) from different habitats in Croatia, European Journal of Wildlife Research, 2004, 50, 7-12.
14. **Ranucci, S., Morgante, M., Diverio, S., Costarelli, S., Beghelli, D., Duranti, E.,** Blood constituents and clinical findings in captured fallow deer (*Dama*

dama), Supplemento alle ricerche di biologia della selvaggina, 1996, 25, 209-217.
15. **Thompson, K.A., Lamberski, N., Rayburn, M., Chigerwe, M.,** Validation of immunocrit as a diagnostic test to detect failure of passive transfer of immunity in hand-reared nondomestic ruminat neonates, Journal of Zoo and Wildlife Medicine, 2019, 50, 3, 627-633.
16. **Tîrziu, E.,** Teză de doctorat, USAMVB Timișoara, 1998.
17. **Tîrziu, E., Trif, R., Vior, C., Cumpănăşoiu, C.,** Metode de diagnostic imunologic, 2002, Ed. Waldpress, Timisoara.
18. **Yuan, S., Tao, X., Huang, S., Chen, S., Xu, A.,** Comparative Immune Systems in Animals, Annual Review of Animal Biosciences, 2014, 2, 235-258.
19. **Zochowska-Kujawska, J., Kotowicz, M., Sobczak, M., Lachowicz, K., Wojcik, J.,** Age-related changes in the carcass composition and meat quality of fallowdeer (*Dama Dama*), Meat Science, 2019, 147, 37-43.
20. ***https://www.merckvetmanual.com/immune-system/the-biology-of-the-immune-system/adaptive-immunity-in-animals.

THE SURVEY OF THE ISOLATION FREQUENCY OF *CAMPYLOBACTER* SPP. IN POULTRY ORIGIN SAMPLES – A PRELIMINARY STUDY

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Summary

The food-borne origin *Campylobacter* spp. infections are recognized as the main cause of gastroenteritis in humans, worldwide. The consumption of undercooked poultry or other cross contaminated foodstuffs are considered mains sources of human campylobacteriosis. Considering the importance of the monitoring of this important food-borne pathogen within the entirely food chain, the present study aimed to provide data on the frequency of isolation of *Campylobacter* spp. in poultry origin samples collected from slaughterhouse, as well as retail level. A total of 48 samples were collected, consisting in neck skin sample mass (n=15) and fresh cecal samples (n=23) from healthy slaughtered broiler chickens in two slaughterhouses of Timiș and Hunedoara counties, as well as from retail raw chicken meat (n=10) sold directly to the consumer in the aforementioned counties. The sampling methodology was based on the requirements of European Union Regulation no. 2017/1495, regarding the presence of *Campylobacter* in chicken samples. Overall, *Campylobacter* spp. was identified in 39.6% (19/48, 95% CI 27.0 – 53.7) of the examined samples, with a distribution of 20.0% (2/10) in the raw meat, 47.8% in the cecal samples, and 40.0% (6/15) at the neck skin level, respectively. The study results strengthen the fact that chicken origin samples constitute an important *Campylobacter* reservoir, and, at the same time, open the opportunity for further epidemiological studies based on molecular characterization, as well as on the monitoring of phenotypic and genotypic antimicrobial resistance profile of the isolated strains.

Keywords: *Campylobacter*, bacteria, isolation, frequency, poultry.

Foodborne diseases produced by *Campylobacter* spp. are among the four main causes of gastroenteritis worldwide. The infection frequency with campylobacteria shown an increasing trend in the last 10 years (3).

Campylobacteriosis represent the most commonly reported zoonosis in developed and low incoming countries. In the European Union, the number of confirmed and reported cases of human campylobacteriosis was more than 240.000 in 2019, representing 66.3 cases per 100,000 population (20). The most frequently isolated *Campylobacter* spp. was *C. jejuni* followed by *C. coli* (14). The increasing identification of emerging pathogens requires a better understanding of how these underappreciated species cause disease, transmit and evolve in humans (4).

Currently, *Campylobacter* genus includes 31 species (4). Out of them, *C. coli* and *C. jejuni* are the most incriminated species in human campylobacteriosis (2).

Most of the species have been isolated from terrestrial and marine mammals, reptiles, domestic and wild birds (12).

Campylobacter spp. are divided into: catalase positive species (*C. fetus*, *C. jejuni*, *C. coli*, *C. lari*) and catalase negative species (*C. sputorum*, *C. concisus*).

Catalase-positive species have been incriminated in infertility cases, abortion, and dysentery in animals. In humans campylobacteria can produce enteritis and bacteraemia. Among these, thermotolerant species are most often involved in human foodborne diseases, the source of infection usually being the consumption of raw or insufficiently heat-treated animal origin foodstuffs especially poultry (1).

In Europe, in 2018, over 250,000 confirmed cases were reported, representing more than 50% of all zoonotic infections in humans. However, these values are likely to be underestimated, the real incidence being probably much higher. Many cases of campylobacteriosis are sporadic, but diseases can also occur in the form of collective episodes (6).

The most important reservoir of campylobacteria is represented by poultry, and the consumption of meat, especially poultry meat may represent a potential risk of campylobacteriosis in humans (11). Over the past decade, a number of control measures have been implemented in several states to reduce the incidence of this foodborne pathogene. In the European Union, Regulation No. 2073/2005 was recently amended by including a new microbiological hygiene criterion *Campylobacter,* the maximum permitted level being 1000 cfu/g in chicken meat (10).

In Romania, the isolation frequency of *Campylobacter* spp. was almost 30% in chicken meat samples. Furthermore, 80% of the tested strains showed resistance to ciprofloxacin and nalidixic acid, 40% to tetracycline and 10% to streptomycin and erythromycin, respectively, which is a real concern for public health (15, 17).

Materials and methods

Sample collection. Between February and April 2022, a total number of 48 skin samples were collected from the neck region and cecums from broiler chickens, from slaughterhouses (named A and B) and 10 retail units located in Timișoara and Hunedoara counties. The samples came from two slaughterhouses (marked A and B) and 10 sales units.

Thus, of the 48 chicken meat samples, 23 samples were represented by cecums (slaughterhouse A), and 15 skin samples were collected from the neck region (slaughterhouse B). A total of 10 samples originated from retail level units, consisting of necks and backs (with skin).

The daily slaughter capacity in slaughterhouse A was between 10,000 and 12,000 birds and broilers were slaughtered at the age of 32 days.

The samples collected from slaughterhouse A (Fig. 1) came from the gastrointestinal mass collected from the batch of broilers, consisting of terminal portions of the cecum, which were subsequently tied to avoid leakage of the intestinal

contents (Fig. 2). The randomly collected samples were placed in sterile bags and transported under refrigerated conditions to the „Laboratory of Microbiology Risk Surveillance" wich is part of „Horia Cernescu Laboratory Complex" (H.C.L.C) of the Faculty of Veterinary Medicine, Timișoara.

Fig. 1. Slaughter line from abattoir

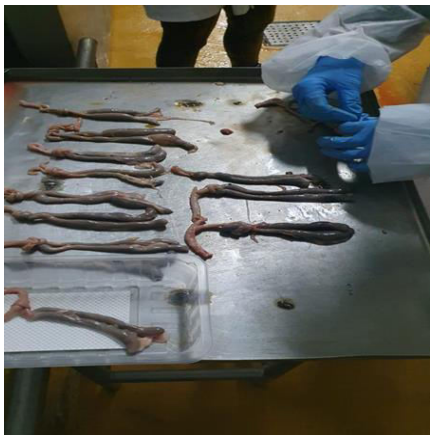

Fig. 2. Tying cecum samples

Whole carcasses samples of the birds were collected from slaughterhouse B, (after cooling) consisting of the sectioned skin around the neck. Samples were collected under aseptic conditions and transported to the laboratory for analysis.

Each sample collected from the 10 sales units were represented by 5 necks and a back, specific to the refrigerated packaged product.

Isolation and identification of Campylobacter spp. from cecum and neck skin samples. The detection and identification of commensal *Campylobacter* spp. strains were performed by direct plating according to the ISO 10272-1:2017 standard (18) and the following selective agars were used: Charcoal Cefoperazone Deoycholate (mCCDA) (Oxoid Ltd.) and Butzler (Oxoid Ltd., Basingstoke, UK). Firstly, a small incision was performed at the cecum level, and a loop of intestinal content was taken and streaked onto the selective agars. Thus, each sample was streaked on a Petri plate containing mCCD agar and, separately, on the surface of another agar plate (Butzler).

The prepared plates were inserted into the jar for incubation. To ensure microaerophilic condition for the development of bacteria of the genus *Camylobacter* (O_2-5%, CO_2- 10% and N_2 -85%), CampyGen™ (Thermo ScientificTM, Waltham, MA, USA) bags were used.

Subsequently the samples were incubated at 41.5°C, in microaerophilic atmosphere, for 44 hours. After incubation, typical colonies (based on colony morphology and characteristic microscopic appearance) were selected for

identification. Each selected colony was streaked onto a non-selective Columbia blood (Oxoid Ltd.) agar plate, then incubated at 41.5°C for 24 hours in a microaerobic atmosphere in order to obtain pure colonies.

The bacteria were identified according to their morphology, motility and growing capacity at 25°C. Other specific biochemical characteristics of presumed *Campylobacter* spp. isolated were studied including oxidase (oxidase detection strips, Oxoid Ltd.) and catalase tests.

The sampling method for isolation and identification of *Campylobacter* spp. from neck skin samples was made in accordance with Reg. (EC) no. 2017/1495 (19), regarding the presence of *Campylobacter* in chicken carcasses.

From slaughterhouse B, 15 neck skin samples were collected randomly, after the cooling stage. For a collective sample of 26 g, three neck skin samples (from three broilers) were selected, which were collected together in the same sterile bag.

Finally, five collective neck skin samples from 15 birds (5 x 26 g) were obtained. After sampling, the samples were transported to the laboratory, under refrigerated conditions. A volume of 234 ml of buffered peptone water (non-selective pre-enrichment medium) at room temperature was added to the 26 grams of skin samples. This mixture was homogenized with a stomacher for about one minute. A volume of 10 ml each from the initial suspensions was transferred into sterile test tubes with selective medium. Thus, a ring loop was taken from the culture obtained in the pre-enrichment medium, which was streaked onto the surface of the mCCD agar plate. The plates were incubated in a microaerobic atmosphere, at 41.5°C, for 24 hours.

For *Campylobacter* spp. detection from the samples collected from the retail units, the processing of samples and isolation method was the same as the one described above.

Samples that developed typical colonies, wet-appearing, gray colonies with a metallic sheen on mCCD agar were considered positive.

Results and discussions

In the detection of *Campylobacter* spp. the results obtained from the microbiological analysis of the processed samples are shown in Table 1 and Table 2.

Out of 48 samples collected from two slaughterhouses (A and B) and from 10 retail units (supermarket type), respectively, 19 samples were *Campylobacter* positive.

A total of 23 samples were collected from the cecum level (slaughterhouse A) and 11 (47.8%) were positive. Approximately half of the slaughtered birds were infected with campylobacteria, at the intestinal level. The high isolation frequency of the *Campylobacter* spp. from cecum samples reflects the important potential of meat contamination with these microorganisms.

Table 1

The prevalence of *Campylobacter* spp. in cecum samples

Samples origin	No. of samples	Results	
		Positive (%)	Negative (%)
Slaughterhouse A	23	11 (47.8%)	12 (52.2%)

Hue et al. (7) in a study conducted in 2011, have found similarity between the rate of cecal contamination and chicken meat infected with campylobacteria, at the slaughterhouse level. Out of 425 carcasses samples, 322 were positive for *Campylobacter* spp., which represents a contamination of more than 75% of the samples. The same number of samples were tested for the presence of *Campylobacter* spp. at cecum level and 327 (76.9%) were positive. The results demonstrated the presence of Campylobacter in both matrices tested.

In the same study, Oliver Hue et al., concluded that the evisceration is the procedure which contributes the most to the spread of the pathogen. Reducing the contamination rate at cecum level and the compliance of biosecurity measures in the slaughterhouses could be a way to reduce the degree of carcass contamination. Risk management solutions could include showering carcasses after evisceration and implementing culling programs based on herd contamination status *Campylobacter* (7).

The prevalence of *Campylobacter* spp. in Spain was investigated by Perez-Arnedo and Gonzalez-Fandos (13) and the results were similar. The study was carried out on samples from three spanish farms (A, B and C). The broilers have been examined both on the farm and in different processing stages in the slaughterhouse. *Campylobacter* strains have been isolated from dirty transport cages, but also in clean ones, in water and on work tables at the end of the day. The pathogen was detected in all carcass samples from farm C, although it was not detected at the farm level. The results revealed that the infected flocks can be the source of contamination for carcasses. However, cross-contamination during transport and the slaughtering process is also very important (13).

In a survey conducted by Rodrigues et al. (16) 816 samples were collected from 70 brazilian slaughterhouses. The prevalence of *Campylobacter* spp. was 35.8% in chicken carcasses with a distribution of 78.5% *C. jejuni* and *C. coli,* 9.7% (16).

A number of 15 neck skin samples were collected from slaughterhouse B. Out of these, 6 (40%) were positive. At the retail level units, *Campylobacter* spp. isolation rate was 20% (2 positive samples out of the 10 tested). Overall, the isolation frequency of *Campylobacter* spp. in chicken meat was 32%. (Table 2). The results obtained were very close to other surveys conducted in Romania, by Tîrziu et al.

(17). However, to get a real image of the frequency and level of contamination with campylobacteria, monitoring studies on larger samples from various points in the food chain are needed.

Table 2

The prevalence of *Campylobacter* spp. in neck skin samples (slaughterhouse B) and neck and back (retail level)

Samples origin	**No. of samples**	**Results**	
		Positive (%)	**Negative (%)**
Slaughterhouse A	15	6 (40%)	9 (60%)
Retail units	10	2 (20%)	8 (80%)
Total	**25**	**8 (32%)**	**17 (6%)**

Garin et al. (5) demonstrated a *Campylobacter* spp. prevalence of 65.5% (491/750) in skin samples from the neck region collected from slaughterhouses or food markets in 5 major cities located in Africa, Oceania, the Indian Ocean and Asia. In Yaounde and Noumea, high detection rates were found (92.7% and 96.7%, respectively). In the samples collected from slaughterhouse, a significantly lower number of contamination with campylobacteria was found compared to samples from direct cutting, by the seller in the market (5).

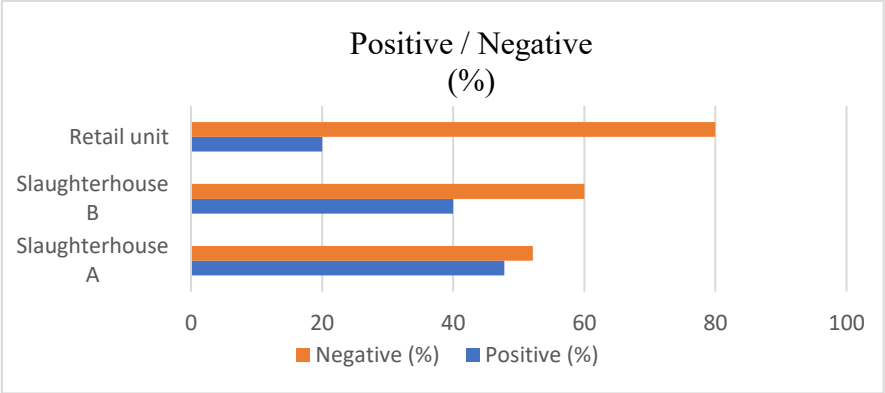

Fig. 1. *Campylobacter s*pp. isolation frequency (slaughterhouses and retail units)

Similar results were reported by Kottawattagw et al. (8) in a survey in which they highlighted the contamination of broiler chicken meat, sold in various retail units.

Neck skin samples were collected from poultry processing facilities (n = 102) and food markets (n = 25). In addition, chicken meat purchased from various retail outlets was tested (n = 37). Prevalence of broiler chicken flocks colonized with campylobacteria was 67%. In both processing facilities and markets, neck skin contamination was found in proportions of 27.4% and 48%, respectively. Molecular characterization of the isolates revealed a higher proportion of *C. coli* compared to *C. jejuni* (8).

Licai Ma et al. (9) assessed the prevalence and distribution of *Campylobacter* spp. along the chicken production chain, from farm to retail. The survey was carried out in a closed-loop farm and abattoir in Shanghai, China and the results showed a 72% prevalence of *Campylobacter* spp. in the samples collected from the cecum and 34.1% in the carcasses samples. In addition, 128 whole carcasses were randomly collected from two supermarkets and the prevalence of campylobacteria was 31.3% (40/128). The results indicated that the isolates of *Campylobacter* spp. isolated from chicken meat from retail units had faecal contamination from the slaughterhouse as their source, and reflected the need of applying measures to reduce carcass contamination at the slaughterhouse level (9).

Conclusions

Increased frequency of isolation rates at the slaughterhouse level reflects a fecal contamination of the poultry meat, which requires the application of measures to improve hygiene on the technological flow.

Maintaining a relatively high rate of contamination of poultry meat with *Campylobacter* in retail outlets is a public health concern.

Reducing the rate of intestinal contamination of birds, at farm level, could be a way to reduce the frequency of carcass contamination.

Observance of hygiene conditions on the flow of cutting birds in the slaughterhouse and avoiding cross-contamination, especially at the evisceration stage, could contribute to reducing the contamination rate of chicken carcasses.

To get a real overview of the frequency and level of contamination with *Campylobacter* spp. monitoring studies on larger samples from various points of the food chain are needed.

References

1. **Bârzoi, D., Apostu, S.,** Microbiologia produselor alimentare, Ed., Risoprint Cluj, 2002.
2. **Butzler, J.P.,** *Campylobacter*, from obscurity to celebrity, Clinical Microbiology and Infection, 2004, 10, 868-876.
3. **Costa, D., Iraola, G.,** Pathogenomics of emerging *Campylobacter s*pecies, Clinical microbiology reviews, 2019, 32, 4, e00072-18.

4. **García-Sánchez, L., Melero, B., Rovira, J.,** *Campylobacter* in the food chain, Advances in Food and Nutrition Research, 2018, 86, 215-252.
5. **Garin, B., Gouali, M., Wouafo, M., Perchec, A.M., Thu, P.M., Ravaonindrina, N., Pouillot, R.,** Prevalence, quantification and antimicrobial resistance of *Campylobacter* spp. on chicken neck-skins at points of slaughter in 5 major cities located on 4 continents, International journal of food microbiology, 2012, 157, 1, 102-107.
6. **Hansson, I., Tamminen, L.M., Frosth, S., Fernström, L.L., Emanuelson, U., Boqvist, S.,** Occurrence of *Campylobacter* spp. in Swedish calves, common sequence types and antibiotic resistance patterns, Journal of Applied Microbiology, 2021, 130, 6, 2111-2122.
7. **Hue, O., Allain, V., Laisney, M.J., Le Bouquin, S., Lalande, F., Petetin, I., Chemaly, M.,** *Campylobacter* contamination of broiler caeca and carcasses at the slaughterhouse and correlation with *Salmonella* contamination, Food Microbiology, 2011, 28, 5, 862-868.
8. **Kottawatta, K.S., Van Bergen, M.A., Abeynayake, P., Wagenaar, J.A., Veldman, K.T., Kalupahana, R.S.,** *Campylobacter* in broiler chicken and broiler meat in Sri Lanka: Influence of semi-automated vs. wet market processing on *Campylobacter* contamination of broiler neck skin samples, Foods, 2017, 6, 12, 105.
9. **Ma, L., Wang, Y., Shen, J., Zhang, Q., Wu, C.,** Tracking *Campylobacter* contamination along a broiler chicken production chain from the farm level to retail in China, International journal of food microbiology, 2014, 181, 77-84.
10. **Myintzaw, P., Jaiswal, A.K., Jaiswal, S.,** A Review on Campylobacteriosis Associated with Poultry Meat Consumption, Food Reviews International, 2021, 1-15.
11. **Oberheim, J., Höser, C., Lüchters, G., Kistemann, T.,** Small-scaled association between ambient temperature and campylobacteriosis incidence in Germany, Scientific reports, 2020, 10, 1, 1-12.
12. **Parisi, A., Chiara, M., Caffara, M., Mion, D., Miller, W. G., Caruso, M., Zanoni, R.G.,** *Campylobacter vulpis* sp. nov. isolated from wild red foxes, Systematic and Applied Microbiology, 2021, 44, 3, 126204.
13. **Perez-Arnedo, I., Gonzalez-Fandos, E.,** Prevalence of *Campylobacter* spp. in poultry in three Spanish farms, a slaughterhouse and a further processing plant, Foods, 2019, 8, 3, 111.
14. **Popa, S.A., Morar, A., Ban-Cucerzan, A., Imre, K.,** Last decade mini-review of the scientific progresses in the monitoring of the occurrence and antimicrobial susceptibility profile of poultry origin Campylobacter spp. within the European Union countries, Revista Română de Medicină Veterinară, 2022, 32, 75-82.
15. **Popa, S.A., Morar, A., Ban-Cucerzan, A., Tîrziu, E., Herman, V., Sallam, K.I., Morar, D., Acaroz, U., Imre, M., Florea, T., Mukhtar, H., Imre, K.,** Occurrence of *Campylobacter* spp. and Phenotypic Antimicrobial Resistance Profiles of

Campylobacter jejuni in Slaughtered Broiler Chickens in North-Western Romania, Antibiotics, 2022, 11, 12, 1713.

16. **Rodrigues, C.S., Armendaris, P.M., de Sá, C.V.G.C., Haddad, J.P.A., de Melo, C.B.,** Prevalence of *Campylobacter* spp. in chicken carcasses in slaughterhouses from south of Brazil, Current Microbiology, 2021, 78, 6, 2242-2250.
17. **Tîrziu, E., Bărbălan, G., Morar, A., Herman, V., Cristina, R.T., Imre, K.,** Occurrence and antimicrobial susceptibility profile of *Salmonella* spp. in raw and ready-to-eat foods and *Campylobacter* spp. in retail raw chicken meat in Transylvania, Romania, Foodborne Pathogens and Disease, 2020, 17, 8, 479-484.
18. ISO 10272-1:2017; Microbiology of the Food Chain—Horizontal Method for Detection and Enumeration of Campylobacter spp.— Part 1: Detection Method. International Organization for Standardization: Geneva, Switzerland, 2017.
19. Regulamentul (UE) 2017/1495 de modificare a Regulamentului (CE) nr 2073/2005 în ceea ce privește *Campylobacter* în carcasele de pui pentru îngrășare. JO L. 218, 24.8.2017, 1-6.
20. *** EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), (2021), The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2018/2019. EFSA Journal, 2021, 19, 4, 6490.

PRELIMINARY STUDY OF THE ISCHIOPUBIC SYMPHYSIS THROUGH PELVIC RADIOGRAPHIC INVESTIGATIONS IN THE AFRICAN PYGMY HEDGEHOG (*ATELERIX ALBIVENTRIS*) FEMALES

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Summary

The potential risk of fusion in the ischiopubic symphysis of the female African hedgehogs that have never been bred before the age of 1.5 years is mentioned in the literature. However, no study was conducted in order to prove this issue. The objective of this study was to show if age in correlation with parity has an influence on the fusion of the ischiopubic bones in the African pigmy hedgehog. The study was performed on sexually mature African hedgehog females. Both nulliparous and multiparous females were studied. Four categories (C1-C4) were constituted having the age and parity as criteria: C1, nulliparous and younger than 1.5 years; C2, multiparous and younger than 1.5 years; C3, nulliparous and older than 1.5 years; C4, multiparous and older than 1.5 years. The *Atelerix albiventris* females were anesthetized to perform the imagistic investigations on their pelvic region. In order to carry out the radiographies the animals were placed in ventrodorsal recumbency with their limbs stretched. The results of the study showed the fusion of ischiopubic symphysis in the hedgehog older than 1.5 years with no history of breeding, while in the other categories of hedgehogs, fusion was not encountered.

Keywords: African pigmy hedgehog, ischiopubic symphysis, nulliparous, multiparous, pelvic radiographs.

African hedgehogs (or four-toed hedgehogs) are small mammals whose dorsal surfaces are covered in a dense coat of several thousand smooth spines (5). There are 17 species in five genera of hedgehogs worldwide (19). Hedgehogs were classified as insectivores before the order *Insectivora* was split in two, putting hedgehogs in the new *Erinaceomorpha* order (5). They are considered relatively primitive mammals (12). Most species of hedgehog have five toes on each foot, except for the four-toed hedgehog, which has only four toes on the back feet (18). There are four species of *Atelerix* distributed across the continent of Africa (9). Contrary to some popular notions, hedgehogs are not related to porcupines (rodents) or echidnas (monotremes) (10).

Hedgehogs are native to West and Central Africa (8). They are found in a variety of habitats, including deserts, steppes, and forests (11). They are nocturnal and spend their daylight hours hidden in burrows or other cavities. They are territorial and solitary except during courtship and when raising offspring (1, 3, 20).

African hedgehogs are polyestrous and breed throughout the year in captivity (15). Gestation is between 34 and 37 days and the average litter size is three (13). Passive transfer of immunity occurs through colostrum in the first 24 to 72 hours of life (9).

Dystocias in hedgehogs are poorly described in the literature and are believed to be rare and uncommon. In theory, the same potential causes for dystocia exist as for other species: uterine inertia or disease, malformation of the reproductive tract, an abnormally large or malpositioned fetus, and so on (2, 17). Possible pelvic symphyseal fusion has been reported as potential dystocia if female hedgehogs are not bred by 1.5 years of age. However, some animals were seen breeding later without complications (7).

The objective of this study was to show if age in correlation with parity has an influence on the fusion of the ischiopubic bones in the African pigmy hedgehog.

Materials and methods

The study was performed on 4 sexually mature African hedgehog females, with ages varying between 11 months and 3 years old. The animals were housed in opened plastic storage boxes, equipped with hiding places where they sleep during the day, food and water dishes, and running wheels. Their diet consisted of kitten dry kibble.

Four categories (C1-C4) were constituted having the age and parity as criteria: C1, nulliparous and younger than 1.5 years; C2, multiparous and younger than 1.5 years; C3, nulliparous and older than 1.5 years; C4, multiparous and older than 1.5 years.

Initially, we tried to perform the radiographic investigations without the use of anesthesia, but it resulted in being unsuccessful even in the tame females. Therefore, to obtain the radiographs of their pelvic region the females were anesthetized using the following anesthetics: Medetomidine 1% sol (Domitor, Orion Pharma) and Ketamine 10% sol (Ketamidor, Richter Pharma AG). Injectable anesthesia was performed by IM way, administering in the rear limb the combination of 0.1 mg/kg Medetomidine and 5 mg/kg Ketamine. For one female, the oldest, in order to achieve immobility and muscle relaxation inhalation anesthesia was necessary (Fig. 1). For this was used anesthesia chamber for small animals with a flow of 1 L/min of 5% isoflurane in oxygen, where female was kept for 5 minutes approximately.

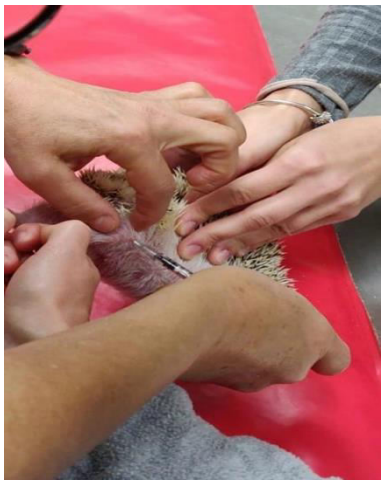
A   B

Fig. 1. Administration of intramuscularly (A) and inhalation (B) anesthesia in the hedgehog females

The subjects were positioned on the radiology table in ventrodorsal recumbency with their hind limbs extended caudally (Fig. 2). The radiological device used was a Siemens Multix Swing (Siemens AG, Germany). Following the radiographic study, the hedgehogs were placed in a quiet, warm environment until fully recovered from the anesthesia. The radiographs were examined for the identification of ischiopubic symphysis.

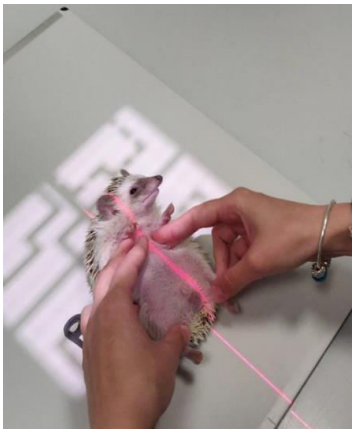


Fig. 2. Hedgehog positioned for a ventrodorsal radiograph of the pelvis

The clinical phase was conducted also to determine if the fusion of the ischiopubic symphysis has any influence over the gestation or parturition process. The nulliparous female older than 1.5 years was put in one cage with another female (who already gave birth 2 times before) and a male. They were kept together for 10 days, then the male was moved away. The gestation and parturition were monitored.

Results and discussions

Even though it is considered to be challenging in hedgehogs, there were no complications following anesthesia. For the majority of females, the administration of Medetomidine and Ketamine was sufficient to keep them properly positioned while taking the radiographs. The chemical restraint and muscle relaxation that allowed the hedgehog to be positioned without reaction on its part, were obvious in approximately 3-5 minutes. Approximately 10 minutes after the procedure the animals were partially awake and they had an easy full recovery.

The three components of the pelvis can be seen on the radiographs: ilium, ischium and pubis (4). Some morphological findings were observed. The wing of the ilium has a vertical orientation, while the *tuber coxae* is proeminent. The *foramenum obturatum* has the base facing craniolaterally (Fig. 3).

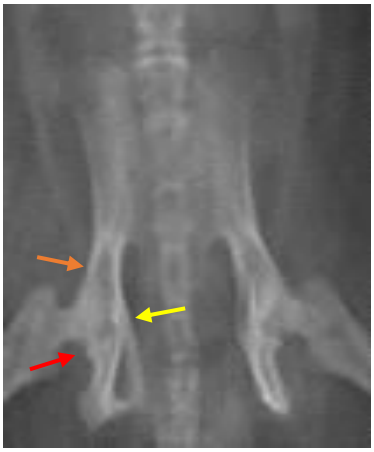
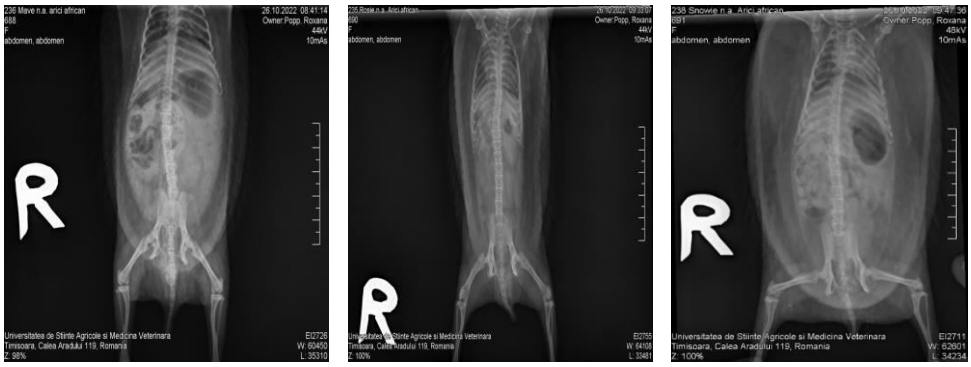


Fig. 3. Identification of the pelvic bones (orange arrow: ilium; yellow arrow: pubis; red arrow: ischium)

The radiological exams of the four females highlighted that neither category 1 nor category 2 or 4 showed any ossified structures at the level of the ischiopubic symphysis (Fig. 4).



A B C

Fig. 4. Ventrodorsal radiographs of the pelvis on females belonging to category 1 (A), category 2 (B), and category 4 (C)

At the radiological investigations in the nulliparous female that was older than 1.5 years, a structure connecting the caudal part of the ischial plate was present (Fig. 5).

The female gave birth 46 days away from the first encounter with the male. The litter consisted of three hoglets. There was no evidence of gestation impairment or dystocia correlated with the presence of the fusion of pubic symphysis.

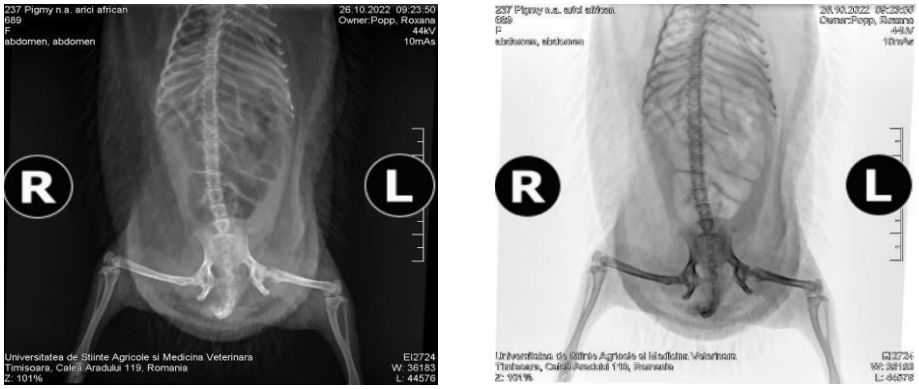


Fig. 5. Ventrodorsal radiograph of the pelvis on the female that presents the fusion of ischiopubic symphysis

Dystocias in hedgehogs, as in most small mammals were considered uncommon because in most of these species, large numbers of small sized offspring

are normally born. They have multiple causes, including the fusion of pelvic symphysis (21).

Another species in which age in correlation with parity has been described as having an influence on the fusion of the pelvic symphysis is the guinea pig (Cavia porcellus). It was reported that sows should be bred before 6–7 months of age to prevent permanent fusion (8). It was written by some authors that impending parturition is signaled by separation of the pubic symphysis; this separation may be inadequate in sows that are bred for the first time after 7 or 8 months of age, and it increases the risk of dystocia (14, 16, 22). Nevertheless, in a recent study, no evidence was found for the ossification of the pubic symphysis in either nonbreeding or breeding female guinea pigs (6).

Our study contributes to the existing knowledge by supporting the theory that normal parturition can occur in female hedgehogs breeding for the first time around 1.5 years of age, even if the pubic symphysis fuses. We consider that the studies on this topic should be continued, in order to know if: - fusion in the pelvic symphysis occurs in all females that did not breed until the age of 1-1.5 years or only in some females; - fusion in the pelvic symphysis is not correlated with dystocia or causes dystocia in some females and what are the coexisting factors that lead to dystocia in this situation; -there are structural and functional changes of the fused pelvic symphysis, during pregnancy and parturition (21).

Conclusions

The radiological investigations revealed the presence of a fusion in the pelvic symphysis of the nulliparous female older than 1.5 years.

The female presenting the fusion of the ischiopubic symphysis had a normal gestation and no complication during the parturition.

References

1. **Banks, R., Sharp, J., Doss, S., Vanderford, D.,** Exotic Small Mammal Care and Husbandry, Willey-Blackwell, Iowa, 2010.
2. **Burgess, M.E., Bishop, C.R.,** Reproductive Physiology, Normal Neonatology, and Neonatal Disorders of Hedgehogs, In: Management of Pregnant and Neonatal Dogs, Cats and Exotic Pets, editors: Lopate, C., Willey Blackwell, Iowa, 2012.
3. **Doss, G.A., Carpenter J.W.,** African Pygmy Hedgehogs, In: Ferrets, Rabbits and Rodents, Clinical Medicine and Surgery, 4th edition, Elsevier, 2021.
4. **Girgiri, I.A, Yahaya, A., Gambo, B.G, Majama, Y.B, Sule, A.,** Osteomorphology of the Appendicular Skeleton of Four-Toed African Hedgehogs (Atelerix albiventris) Part (2): Pelvic Limb, Department of Veterinary Anatomy, University of Maiduguri, Maiduguri, Nigeria, IDOSI Publications, 2016.

5. **Goertzen, K.,** Pet African Hedgehogs, lulu.com, 2012.
6. **Hugon H, Bruyas J.,** Suivi de l'évolution du tissu fibro-cartilagineux de la symphyse pubienne par tomodensitométrie chez des cobayes femelles précocément primipares ou non mises à la reproduction au cours de la première année de vie, Doctoral thesis, Veterinary Medicine, Université de Nantes, 2015.
7. **Ivey, E., Carpenter, J.W.,** African hedgehogs. In: Ferrets, Rabbits, and Rodents: Clinical Medicine and Surgery, 2nd ed., Saunders Publishing, St. Louis, 2004.
8. **Johnson, D.,** African pygmy hedgehogs, In: BSAVA Manual of Exotic Pets, Fifth Edition, British Small Animal Veterinary Association, 2010.
9. **Johnson, D.,** Diagnosing and Treating African Pygmy Hedgehogs, Atlantic Coast Veterinary Conference, 2004.
10. **Judah, V., Nuttall, K.,** Exotic Animal Care and Management, Thompson Dellmar, NY, 2008.
11. **Larson, R.S., Carpenter, J.W.,** Husbandry and medical management of African hedgehogs, Veterinary Medicine, 1999, 94, 877-88.
12. **Nowak, R.M.,** Walker's Mammals of the World, 5th ed, Baltimore and London: The John Hopkins University Press, 1991.
13. **Pinney, C.C.,** Hedgehogs, In The Complete Home Veterinary Guide, Third edition, McGraw - Hill Professional, New York, 2003.
14. **Quesenberry, K.E., Donnelly, T.M., Mans, C.,** Biology, husbandry and clinical techniques of guinea pigs and chinchillas, 3rd edn, St Louis, Elsevier, 2012.
15. **Reeve, N.,** Hedgehogs, London: T & AD Poyser, 1994.
16. **Ruelokke, M.L., Koch, J., Jensen, A.L.,** Diagnostic tools in renal dysfunction in Guinea pig, World Small Animal Veterinary Association, 2006.
17. **Schuller, A., Duffy, M.J.,** Hedgehogs, In: Exotic Animal Medicine for the Veterinary Technician, Wiley Blackwell, Iowa, 2017.
18. **Storer, P.,** Everything You Wanted to Know About Hedgehogs But You Didn't Know Who to Ask, Columbus, TX: Country Store Enterprises, 1994.
19. **Turner, P., Brash, M., Smith, D.,** Pathology of Small Mammal Pets, John Wiley & Sons, Hoboken, 2018.
20. **Tynes, V.,** Behavior of Exotic Pets, Wiley-Blackwell, 2010.
21. **Varga, M., Morris, T.,** Laboratory and Pet Rodents, and Lagomorphs, In:Veterinary Reproduction & Obstetrics, 10th Edition, Elsevier, 2019.
22. **Weir, B.J.,** Notes on the origin of the domestic guinea-pig, Symposia of the Zoological Society of London, 1974, 34, 437-446.

RESEARCH REGARDING PREVALENCE OF CHOLECYSTITIS IN DOGS

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Summary

Canine gallbladder related diseases are being reported with an increased frequency by specialists working in small animal veterinary medicine. Investigations carried out on 186 patients revealed that prevalence is greater with females 62.90% (n=117) and with dogs between the ages of 6 to 10 years old 43.01% (n=80). Most common breed identified with cholecystitis is mixed breed 22.58% (n=42). The majority of patients were brought in because of acute symptomatology - 58.06% (n=108) and the most common identified symptom was emesis, in 76.34% (n=142) of cases. After analyzing the data collected from all the patients 58.06% (n=108) were diagnosed with acute cholecystitis accompanied by cholestasis in 43.51% of cases (n=47), microlithiasis in 29.35% of cases (n=32), comorbidities in 20.37% of cases (n=22) or trauma in 6.48% of cases (n=7). 41.93% (n=78) of the patients included in the research were diagnosed with chronic cholecystitis accompanied by cholestasis in 29.48% (n=23) of cases, gallbladder lithiasis in 12.82% of cases (n=10), comorbidities in 34.61% of situations (n=27), microlithiasis in 11.53 % (n=9) of cases and gallbladder mucocele in 6.41% (n=5) of cases. Chronic cholecystitis was also identified as a consequence of extrahepatic gallbladder obstruction in 5.12 % (n=4) of the cases analyzed. After applying routine diagnostic exams in 64 patients, 32.81% (n=21) of them were found manifesting the disease systemically. Most common identified hematological variations was inflammatory leukogram in 21.87% (n=14). Most often identified changes in biochemical exams were hyperbilirubinemia 9.35% (n=6) and an increase in activity of serum transaminases in 15, 62% (n=10) of patients. Most common identified ultrasonographic changes were mucosal hyperplasia in 81.81% (n=18) of patients and the presence of intraluminal sediment in 72.72% (n=16). Finding of this research can help elaborate diagnostic protocols concerning cholecystitis in dogs and characterize the canine population most at risk.

Keywords: cholecystitis, cholestasis, prevalence.

Canine gallbladder related diseases are being reported with an increased frequency by specialists working in small animal veterinary medicine (17, 20). This fact is viewed as a natural consequence resulting from the evolution of veterinary medicine standards, superior investigation methods, increased concern of pet owners and increased number of routine pet consults and veterinary exams (2, 5, 8, 12). Another factor contributing to the rise of identifying cholecystitis and other gallbladder related diseases in dogs are the changes being made in pet dietary and activity regiments and the personification of pets experienced in this current social context (16, 18, 19). Elements characterizing gallbladder diseases in general and disease evolution tendencies are of great interest to medical practitioners working in

small animal veterinary medicine and research, dog breeders and pet owners (13, 14).

Materials and methods

The research activity was conducted between 2016 and 2022 in Râmnicu Vâlcea on a number of 186 of patients diagnosed with cholecystitis, both female and male, of various breeds, ages and reproductive statuses, as shown in Table 1. The patients were included in the research after confirming the diagnosis with the help of clinical and paraclinical investigations such anamnesis, clinical examination and subsequent hematology, biochemistry and ultrasonography exams. Hematology values being tested were hemoglobin, hematocrit, total white cell count, granulocytes count, lymphocytes count and thrombocytes. Biochemistry values recorded were serum transaminases, bilirubin values, alkaline phosphatase and gamma glutamyltransferase values.

Results and discussions

Summarizing and analyzing the data obtained from this research, alongside with identifying key characteristics of the population most at risk and most common symptoms displayed by it offer valuable resources for clinicians working in small animal medicine and one of the main objectives of this research (9, 15).

Of all the patients submitted for a clinical consult, 562 of them displayed symptoms correlating with diseases of the gastrointestinal tract. After performing clinical consults and additional bloodwork and imagining investigations, 186 dogs were included in the study.

The canine population had individuals of both sexes, with varied hormonal status and ages between one to fourteen years old. The breed of the dogs included in the study were cross breed 22.58% (n=42), Pekingese 3.76% (n=7), Yorkshire terrier 14.51 % (n=27), Beagle 7.52% (n=14) , Golden Retriever 13.97% (n=26), Airedale Terrier 2.15% (n=4), Labrador 13.97 % (n=26), Argentinian Dog 1.07% (n=2), Cocker Spaniel 1.07% (n=2), Cane Corso 0.53 % (n=1) and Malinois 0.53% (n=1).

The results obtained in the prevalence of cholecystitis by breed are shown in Fig. 1.

Table 1

Structure of the group of patients

Patients included in study						
Breed		Age			Sex	Reproductive status
Metis	(n=42)	1-6 years	6-10 years	10-14 years	F=117	Sterilized females =38
Pekingese	(n=7)	(n=38)	(n=80)	(n=68)		Intact females=79
Bichon Maltez	(n=34)				M=68	Neutered males=16
Yorkshire Terrier	(n=27)					Intact males=53
Beagle	(n=14)					
Golden Retriver	(n=26)					
Airedale Terrier	(n=4)					
Labrador	(n=26)					
Dog Argentinian	(n=2)					
Cane corso	(n=1)					
Malinois	(n=1)					
Cocker spaniel	(n=2)					

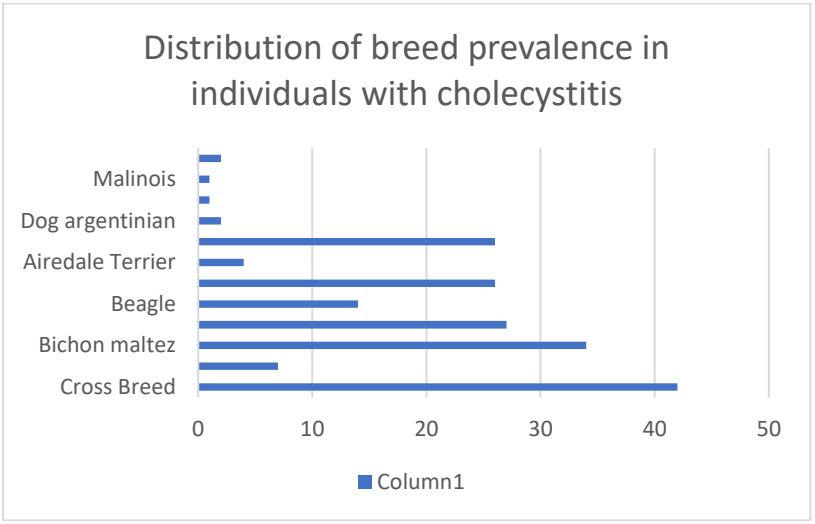

Fig. 1. Distribution of breed prevalence in individuals with cholecystitis

62.90% (n=117) of the patients taking part in the study were female and the remaining 37.47% (n=69), were male. 32.47% (n=38) of the female participants were sprayed and 67.52% (n=79) were not. In the male population, 23.18 (n=16) were neutered and the rest of 76.81 (n=53) were intact. According to the data collected in this study, the prevalence of cholecystitis was greater with intact females, as shown in Fig. 2.

When speaking of the age categories included in the study, patients were included in 3 age groups, the first one being between the ranges of 1-6 years old (n=20.43%), the second group, between 6-10 years old 43.01% (n=80), being the large population sample and the third and final group, with ages between 10-14, comprising of 36.55% (n=68) of the population. These findings are consistent with the data available regarding the prevalence of cholecystitis in dogs (4).

Results concerning the prevalence of cholecystitis by age group are shown in Fig. 3.

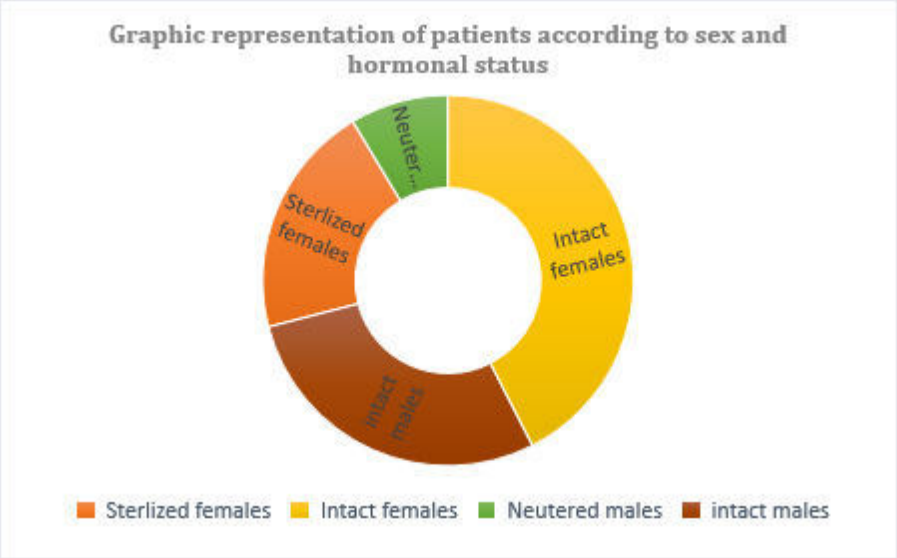

Fig. 2. Graphic representation of patients according to sex and hormonal status

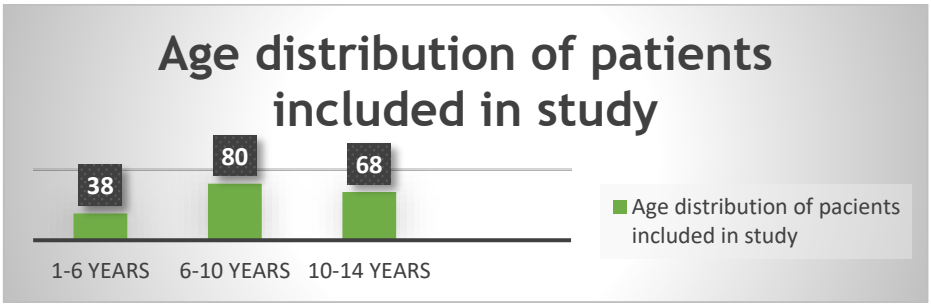

Fig. 3. Figure showing age distribution of patients included in study

Symptoms characterizing cholecystitis can be seen as symptoms displayed by patients suffering from gastrointestinal disease, with nonspecific signs such as inappetence, lethargy, abdominal discomfort, perturbance in stool frequency and consistency and vomiting episodes (1, 3). For some of the patients included in the study, these changes have intervened abruptly and they were brought in shortly after displaying symptoms, in a time frame no longer than 2 days. The proportion of these patients was 58.06% (n=108). The rest of the patients (n=78) started manifesting signs of discomfort earlier on and were brought in because of lack of dissolution or worsening of clinical signs.

Most common symptom was vomiting, in 76.34% (n=142) of cases. 16.90% (n=24) of patients were brought in a time span of 48 hours after one episode of vomiting, 50.70% (n=72), were brought in after 2 or more episodes of vomiting in 3 days and 32.39% (n=46) of the patients presented for a medical consult experiences more than 3 episodes of vomiting in the last week.

The intensity of vomiting episodes experienced by the patients included in this study is shown in Fig. 4.

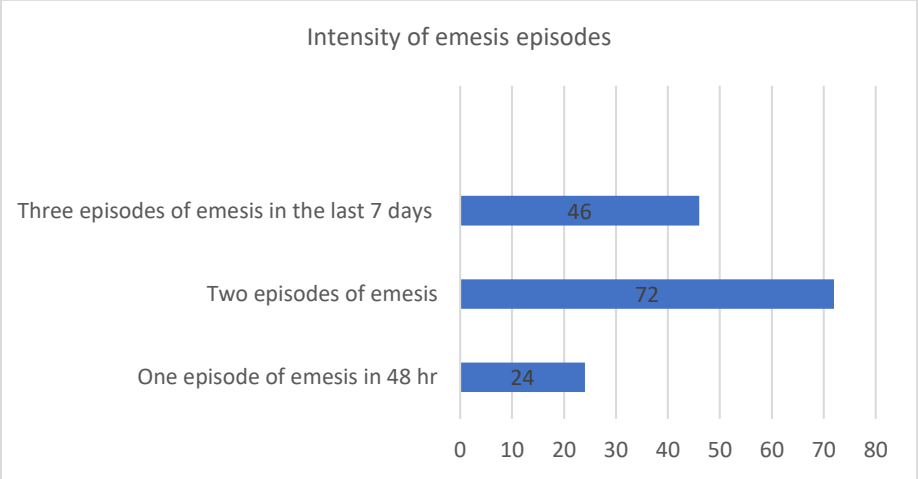

Fig. 4. Frequency of emesis episodes in patients included in research

Second most common symptom found in the patients included in this study was lethargy, in 68.81% (n=128) of patients, followed by abdominal discomfort 43.54% (n=81) and inappetence in 39.24% (n=73) of dogs. 32.79 % (n=61) of the cases submitted reported variations in stool frequency and consistency, as shown in Fig. 5.

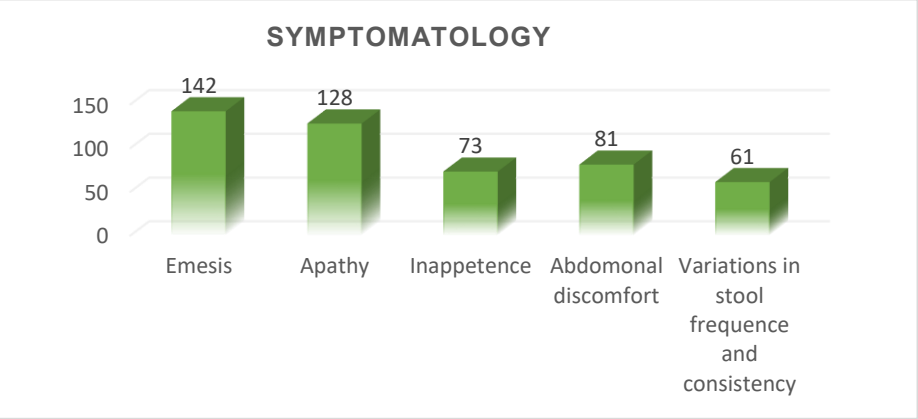

Fig. 5. Graphic representation of symptomatology registered in patients

Variations in body temperature tend to indicate the extent of the body's response to the pathological process at hand and provide an important instrument in assessing the body's capabilities to withstand it. 41% (n=51) of patients experienced low grade fever or fever. 3.76% (n=7) of patients also exhibited icteric mucosae during clinical examinations, displaying signs of advanced pathological processes. Results are summarized in Fig. 6.

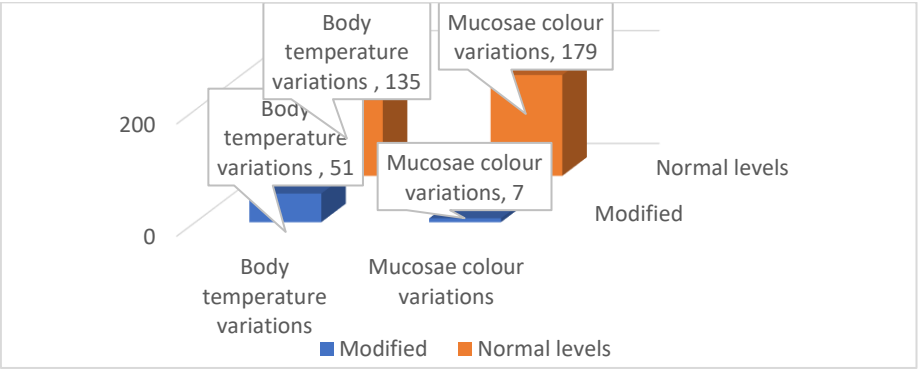

Fig. 6. Variations in body temperature and mucosal coloration in patients with cholecystitis

Out of the 186 patients, 58.06% (n=108) were identified with acute cholecystitis. In 43.51% (n=47) of the cases, it was accompanied by cholestasis, in 29.62 % (n=32) of the cases by microlithiasis, other comorbidities in 20.37% (n=22) of cases and trauma in 0.06% (n=7) of cases. Literature sources also cite

cholecystitis also in cases of renal insufficiency, hyperhydration and canine infectious hepatitis (1). Results are presented in Fig. 7.

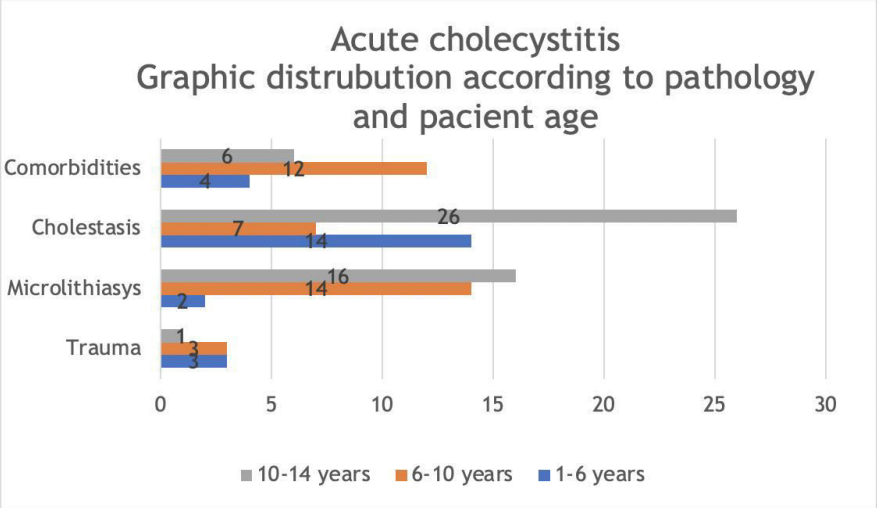

Fig. 7. Graphic representation of identified pathology and age of patients

41.93% (n=78) of the patients included in the study were diagnosed with chronic cholecystitis. Out of them, 29.48% (n=23) of patients were identified with cholestasis, 12.82% (n=10) with lithiasis and 11.53% (n=9) of them also had microlithiasis. 6.41% (n=5) were identified with chronic cholecystitis accompanied by mucocele, 5.12 % (n=4) with extrahepatic obstruction and 34.61% (n=27) with other comorbidities. Results can be seen in Fig. 8.

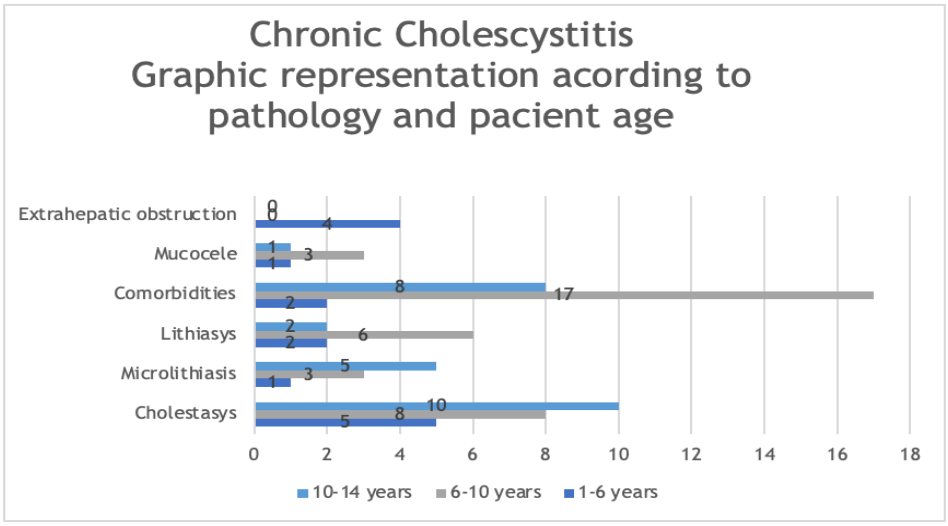

Fig. 8. Graphic representation of pathology and age categories of patients

After analyzing all the data regarding the symptomatology of the parents included in the study, a series of recommendations regarding additional paraclinical testing were made in order to establish a diagnosis.

In this study, 64 blood samples were evaluated. It was concluded that 32.81% (n=21) of patients had some variations of results in hematology, biochemistry or ultrasonography results.

After centralizing the data at hand, main biochemistry variations were hyperbilirubinemia in 9.35% (n=6) of cases and elevated values of serum transaminases in 15.62% (n=10) of cases. Results can be seen in Fig. 9.

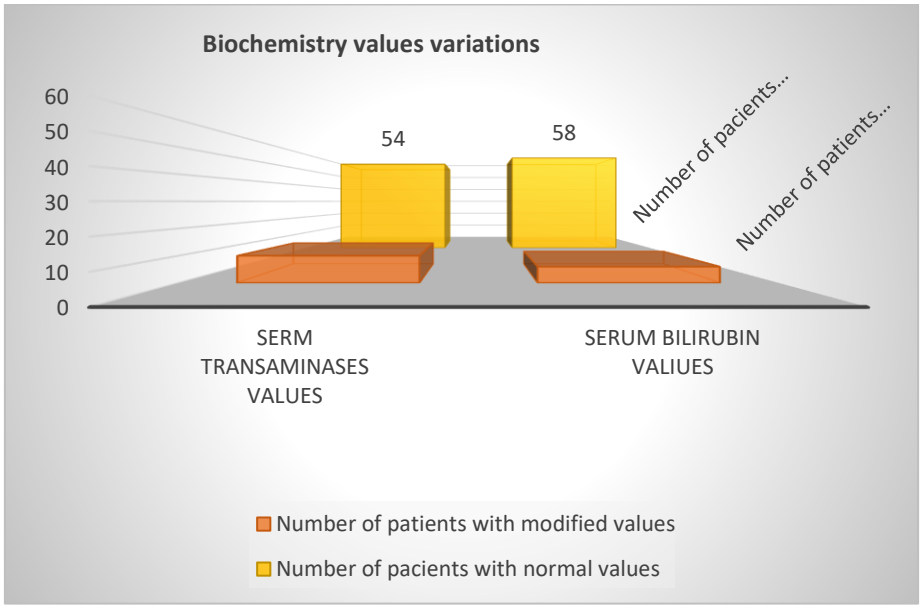

Fig. 9. Graphic representation of number of patients with modified values in biochemical tests

Hematology exams showed that 21.87% (n=14) of patients expressed hematologic changes consistent with inflammatory leukogram. Results are presented below in Fig. 10.

After analyzing data from 22 ultrasonography exams, it was concluded that the most common findings were variations in size of the biliary mucosae 81.81% (n=18) and the presence of intraluminal sediment of mineral collections in 72.72% (n=16). In addition to that, in 27.27 % of cases (n=6), biliary tree hyperplasia could be observed. These findings were aligned with those commonly identified in this sort of condition, according to literary sources (6, 7, 11). Results are summarized in Fig. 11.

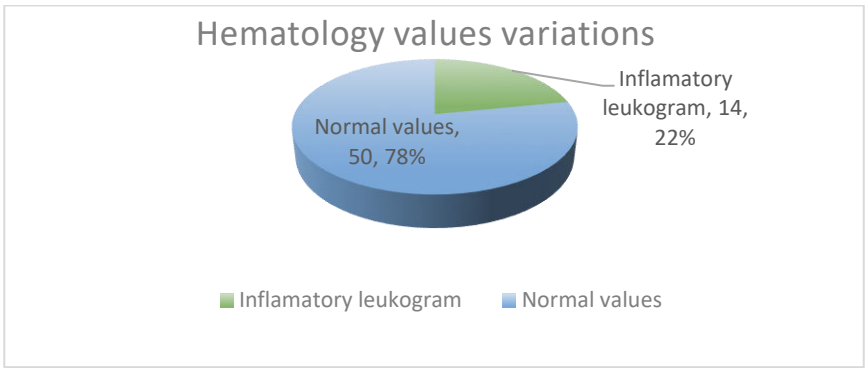

Fig. 10. Graphic representation of number of patients with alterations in hematology exams

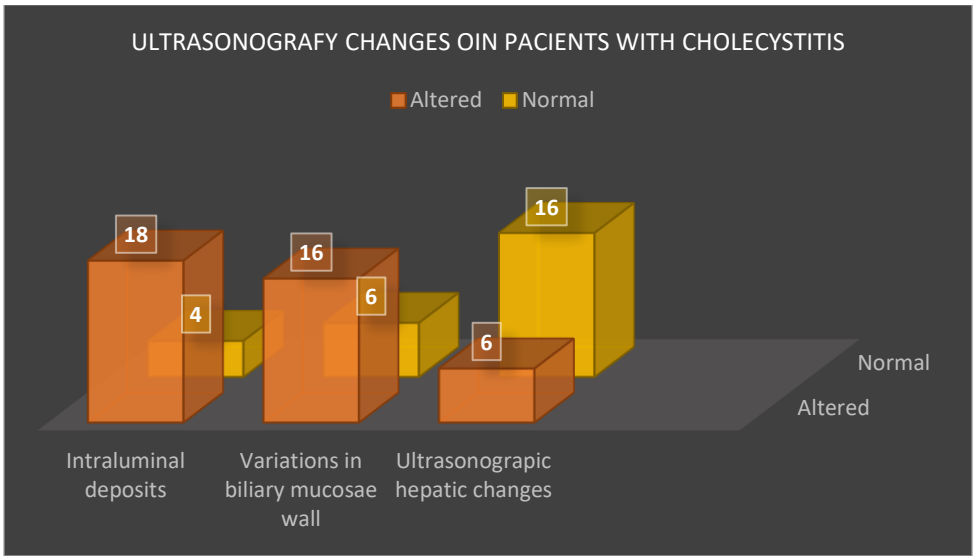

Fig. 11. Graphic representation of number of patcients with alterations in ultrasonography exams

Conclusions

Investigations carried out on 186 patients revealed that prevalence is greater with females 62.90% (n=117) and with dogs between the ages of 6 to 10 years old 43.01% (n=80).

Most common breed identified with cholecystitis is mixed breed 22.58% (n=42).

The majority of patients were brought in because of acute symptomatology, summarizing a total of 58.06% (n=108) and the most common identified symptom was emesis, in 76.34% (n=142) of cases.

After analyzing the data collected from all the patients included in this research, 58.06% (n=108) were diagnosed with acute cholecystitis accompanied by cholestasis in 43.51% of cases (n=47), microlithiasis in 29.35% of cases (n=32), comorbidities in 20.37% of cases (n=22) or trauma in 6.48% of cases (n=7). 41.93% (n=78) of the patients included in the research were diagnosed with chronic cholecystitis accompanied by cholestasis in 29.48% (n=23) of cases, gallbladder lithiasis in 12.82% of cases (n=10), comorbidities in 34.61% of situations (n=27), microlithiasis in 11.53 % (n=9) of cases and gallbladder mucocele in 6.41% (n=5) of the population investigated.

Chronic cholecystitis was also identified as a consequence of extrahepatic gallbladder obstruction in 5.12 % (n=4) of the cases analyzed. After applying routine diagnostic exams in 64 patients, 32.81% (n=21) of them were found manifesting the disease systemically.

Most common identified variations in hematology exams was inflammatory leucograms in 21.87% (n=14).

After examining biochemical exam results, it was concluded that most often identified changes in biochemical exams were hyperbilirubinemia 9.35% (n=6) and an increase in activity of serum transaminases in 15.62% (n=10) of patients.

After conducting ultrasonography exams, most common identified changes were mucosal hyperplasia in 81.81% (n=18) of patients and the presence of intraluminal sediment in 72.72% (n=16).

Finding of this research can help elaborate diagnostic protocols.

Acknowledgement

I would like to thank professor Mario Codreanu for supervision and guidance and doctor Alexandra Mihaela Popa for support.

References

1. **Codreanu, M.D.,** Diagnostic ecografic la bolile interne la animale, Editura Coral Sanivet, București, 2000.
2. **Codreanu, M.D., Crivineanu M.,** Terapeutică Veterinară, Editura Printech, București, 2008.
3. **Codreanu, M.,** Examenul ecografic în diagnosticul colecistopatiilor şi colangiopatiilor la câine şi pisică, Revista Română de Medicină Veterinară, 2002, 12, 1-2, 61-69.
4. **Codreanu, M., Orăşanu, A., Constantinescu, I., Codreanu, I., Rădoi, I.,** Aspecte clinice, hematologice, biochimice şi ecografice la câinii cu afecţiuni hepatobiliare, Al IV-lea Simpozion Aniversar al Institutului de Diagnostic şi Sănătate Animală „35 de ani de activitate în diagnosticul medical veterinar pentru apărarea sănătăţii animale şi umane", 2001, Bucureşti, România.
5. **Ettinger, S., Feldman, E.C.,** Textbook of Veterinary Internal Medicine Consult, Ed. Elsevier- Health Sciences Division, Amsterdam, 2016.
6. **Garcia, D.A., Froes, T.R.,** Importance of Fasting in Preparing Dogs for Ultrasound Examination of Specific Organs, Journal of Small Animal Practice, 2014, 55, 12, 630-634.
7. **Herman, B., Brawer, R.S., Murtaugh R.J., Hackner, S.,** Therapeutic Percutaneous Ultrasound-guided Cholecystocentesis in Three Dogs with Extrahepatic Biliary Obstruction and Pancreatitis, Journal of the American Veterinary Medical Association, 2006, 227, 11, 1782-6.
8. **Kahn, C.M, Line, S.,** Manualul Merck de Medicină Veterinară, ediția a X-a, Editura Callisto, București, 2014.
9. **Lawrence, Y.A.,** Characterization, Treatment, and Outcome of Bacterial Cholecystitis and Bactibilia in Dogs, Journal of the American Veterinary Medical Association, 2015, 246, 9, 982-989.
10. **Lee, S.P, Lamont, J.T., Carey, M.C.,** Role of Gallbladder Mucus Hypersecretion in the Evolution of Cholesterol Gallstones: Studies in the Prairie Dog, Journal of Clinical Investigation, 1981, 67, 6, 1712-1723.
11. **McMaster, P.D., Peyton, R.,** The biliary obstruction required to produce jaundice, Journal of Experimental Medicine, 1921, 33, 6, 731-750.
12. **Mihai, D., Codreanu, M.,** Medicina internă a animalelor, vol.III, Editura Printech, Bucureşti, 2002.
13. **Nakayama, S., Mori, T.,** Effects of distension of the gall bladder and biliary ducts on the movements of the stomach and intestines in the dog, Japanese Journal of Physiology, 1967, 17, 4, 458-465.
14. **Pertsemlidis, D., Kirchman, E.H., Ahrens, E.H.Jr.,** Regulation of Cholesterol Metabolism in the Dog: Effects of complete bile diversion and of cholesterol feeding on absorbtion, syntesis, accumulation and excretion rates measured during life, Journal of Clinical Investigation, 1973, 52, 9, 2353-67.
15. **Rivers, B.J., Walter, P.A., Johnston, G.R., Merkel, L.K., Hardy, R.M.,**

Acalculous cholecystiitis in four cannine cases: ultrasonographic findings and use of ultrasonographic-guided, percutaneous cholecystocentesis in diagnosis, Journal of the American Veterinary Medical Association, 1997, 33, 3, 207-214.

16. **Secchi, P., Pöppl, A.G., Ilha, A., Kunert, Filho, H.C., Lima, F.E., García, A.B., González F.H.,** Prevalence, risk factors, and biochemical markers in dogs with ultrasound- diagnosed biliary sludge, Research in Veterinary Science, 2012, 93, 3, 1185-1189.
17. **Shaffer, E.A.,** Review Article: Control of Gall-bladder Motor Function, Alimentary Pharmacology Therapeutics, 2002, 14, 2, 2-8.
18. **Tamborini, A., Jahns, H., McAlilster, H., Kent, A., Harris, A., Procoli, F., Allenspach, K., Hall, E.J., Day, M.J., Watson, P.J., O'Neaill, E.J.,** Bacterial Cholangitis, Cholecystitis, or Both in Dogs, Journal of Veterinary Internal Medicine, 2016, 30, 4, 1046-1055.
19. **Van Den, I., Ted, S.,** G.A.M. WSAVA Standards for Clinical and Histological Diagnosis of Canine and Feline Liver Disease, Editure Saunders, Philadephia, 2006.
20. **Wagner, K.A., Hartmann, F.A., Trepanier, L.A.,** Bacterial Culture Results from Liver, Gallbladder, or Bile in 248 Dogs and Cats Evaluated for Hepatobiliary Disease: 1998–2003, Journal of Veterinary Internal Medicine, 2007, 21, 417-424.

RESEARCH REGARDING THE USE OF KRILL OIL IN CANINE PATIENTS SUFFERING FROM HYPERLIPIDEMIA

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Summary

Krill Oil is a sustainable source of Omega 3 polyunsaturated fatty acids, rich in eicosapentaenoic acid and docosahexaenoic acid. Salmon oil is another natural source of polyunsaturated fatty acids transported with the aid of triglycerides, whereas krill oil fatty acids are transported using phospholipids and phosphatidylcholine. The benefits of krill oil and salmon oil are being exploited by veterinary clinicians specialized in hyperlipidemic pathology for their ability to regulate cholesterol and triglycerides levels in canine patients. After conducting a statistical analysis on a group of 87 canine patients, divided in 6 groups according to their body conditions score, all of them receiving variable doses of Krill oil and salmon oil, and centralizing the data collected from the analyses, it was concluded that krill oil and salmon oil prove to be efficient in statistically lowering cholesterol and triglycerides level in 83.33% (n=5) of cases. Furthermore, the research shows that doubling the dose of Krill oil does not lead to a greater decrease in cholesterol and triglycerides levels. Krill oil was found to be more effective that salmon oil in lowering serum lipidic values. Nevertheless, both were proved to be efficient in influencing serum lipidic values and can be considered of aid in treating disease associated with hyperlipidemic pathology.

Keywords: hyperlipidemia, krill oil, salmon oil.

Krill Oil is a sustainable source of Omega 3 polyunsaturated fatty acids (11, 13) and choline. Another valuable essential supplement, useful in synthesization of neurotransmitters and phosphocholine, like sphingomyelin. Choline is also necessary in lipid transport and reduction of homocysteine (9). Krill oil has also been used for its anti-inflammatory effects (2, 18), its antiarrhythmic and blood tension reducing properties (1, 4, 12), for the treatment of hyperlipidemia and even premenstrual pain (3, 14, 15, 16, 17, 19, 20). Salmon oil is another natural source of polyunsaturated fatty acids. Monitoring the results of treatment with these nutraceuticals and establishing whether or not they can produce a significant statistical variation in serum lipid values is of great value for the management of diseases associated with hyperlipidemia in dogs.

Materials and methods

The research activity took place between 2016-2022 in Rm Valcea and was conducted on 87 canine patients, male and female, of various ages, breeds and hormonal statuses, as can be seen in Table 1. The patients were divided in 6 groups according to their body condition score and treatment received. They were administered either Krill Oil or Salmon Oil for a period of 80 days, as shown in Table 2. Blood samples were collected from each patient at the beginning of treatment and then at the end of it and the results were analyzed using the statistical procedure called student test. Cholesterol and triglycerides values were measured.

Table 1

Presentation of the study group in relation to breed, age category, sex and hormonal status

Patients included in the study							
Breed	***Age Category***			***Sex***			
	1.5-6 years	**6-10 years**	**10-14 years**	**Male**		**Female**	
				Neutered	**Intact**	**Neutered**	**Intact**
Cross Breed	**9**	**3**	**9**	**3**	**9**	**7**	**2**
Labrador	**1**	**9**	**4**	**1**	**5**	**3**	**5**
Bichon Maltese	**5**	**7**	**1**	**2**	**4**	**1**	**6**
Yorkshire Terrier	**4**	**7**	**1**	**0**	**2**	**6**	**4**
Beagle	**11**	**8**	**0**	**1**	**2**	**8**	**8**
Basset	**2**	**2**	**0**	**1**	**0**	**1**	**2**
Chihuahua	**1**	**1**	**0**	**0**	**0**	**1**	**1**
Pekingese	**0**	**2**	**0**	**2**	**0**	**0**	**0**

Table 2

Dosage of patients according to their body condition score

Dosage Krill Oil/ Salmon Oil			
BCS<3	**Group 1**	**Group 2**	**Group 3**
	17 patients-1 caps (500mg)/10kg/zi	**12 patients-2 caps (1000mg)/10kg/zi**	**16 patients-15ml/10kg/zi**
BCS>3	**12 patients-2 caps (1000mg)/10kg/zi**	**17 patients-4 caps (2000mg)/10kg/zi**	**13 patients-15ml/10kg/zi**

Results and discussions

The participants included in this study were both male and female, neutered/ sterilized and intact and of different ages. 63.21% (n=55) were female and 36.78% (n=32) were male. Out of the females included in the study, 49.09% (n=27) were sterilized and 50.90% (n=28) were not. Out of the males included in the study, 31.25% (n=10) were neutered, and the rest of them, meaning 68.75% (n=22) were intact. Results are summarized in Fig. 1.

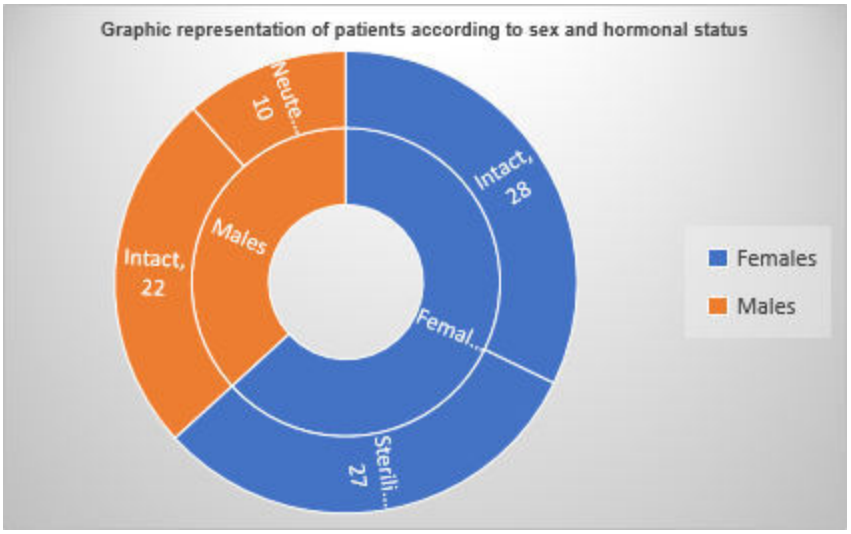

Fig. 1. Graphic representation of population according to sex and hormonal status

Patients were of different breeds, such as Labrador Retriever 16.09% (n=14). Bichon Maltese 14.94% (n=13). cross breed 24.13% (n=21). Yorkshire Terrier 13.79% (n=12). Beagle 21.83% (n=19). Basset Hound 4.59 % (n=4).

Chihuahua 2.29% (n=2). Pekingese 2.29% (n=2). Results are summarized in Figure 2.

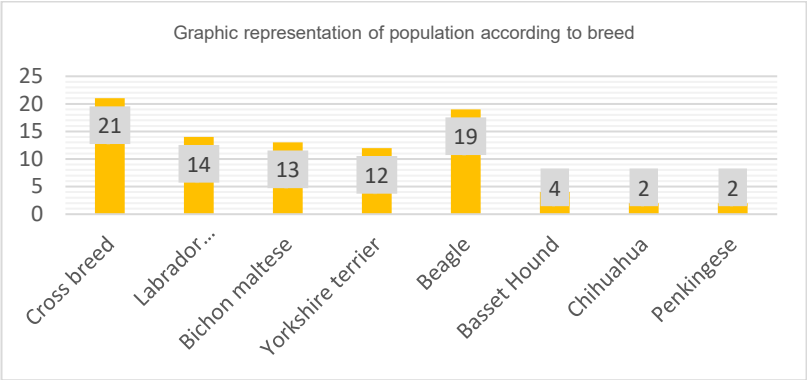

Fig. 2. Graph of population according to breed distribution

Ages of study participants varied. 37.93% (n=33) being in the age group of 1.5-6 years. 44.82% (n=39) in the age group of 6 to 10 years and 17.24% (n=15) of participants were identified as having between 10 to 14 years. Most patients were identified in the 6 to 10 years range, consistent with facts disclosed by medical research done on canine patients with hyperlipidemia (5, 6, 7, 8, 10). Results are summarized in Fig. 3.

All patients had in their medical records events such as clinical examinations due to digestive symptoms, paraclinical investigations and some of them were even hospitalized due to pathological changes related to metabolic diseases in the last 3 months. Out the medical records at hand, it was concluded that 39.08 (n=34) were diagnosed with cholestasis. 19.54% (n=17) with obesity. 19.54% (n=17) with hypothyroidism. 3.44% (n=3) with diabetes. 2.29% (n=2) with Cushing. 16.09% (n=14) with pancreatitis, as shown in Fig. 4. These findings are consistent to previous research done on hyperlipemia and accompanying comorbidities (5, 6, 7, 8, 10, 15, 16).

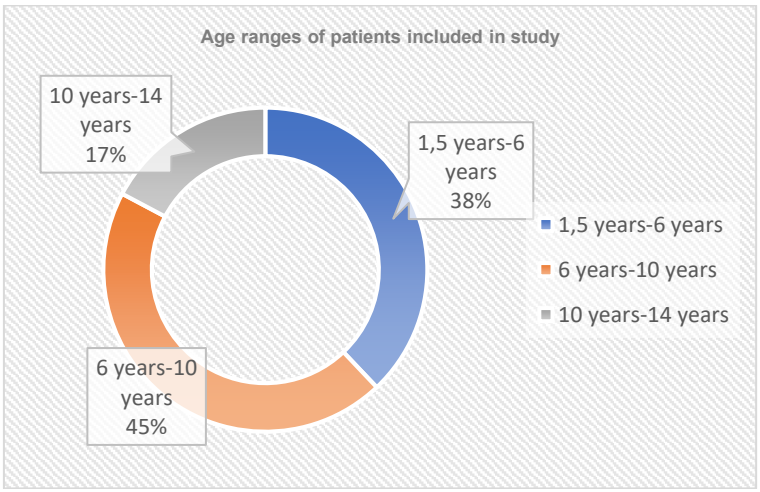

Fig. 3. Graphic representation of population according to age

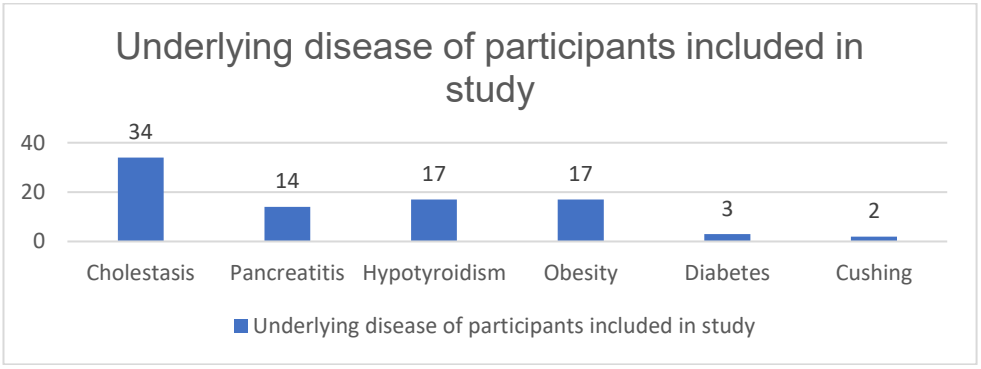

Fig. 4. Graphic representation of underlying disease of patients included in study group

The commercial product Of Krill Oil used contains 500 mg of Krill and the commercial product with fish oil used contains 100% salmon oil. Patients were assigned to study groups in a randomized fashion, except for the criteria of the body condition score. These products were administered to patients according to their body condition scale and their study group. Graphic representation of patients according to repartition to study groups and dosages used is shown in Fig. 5.

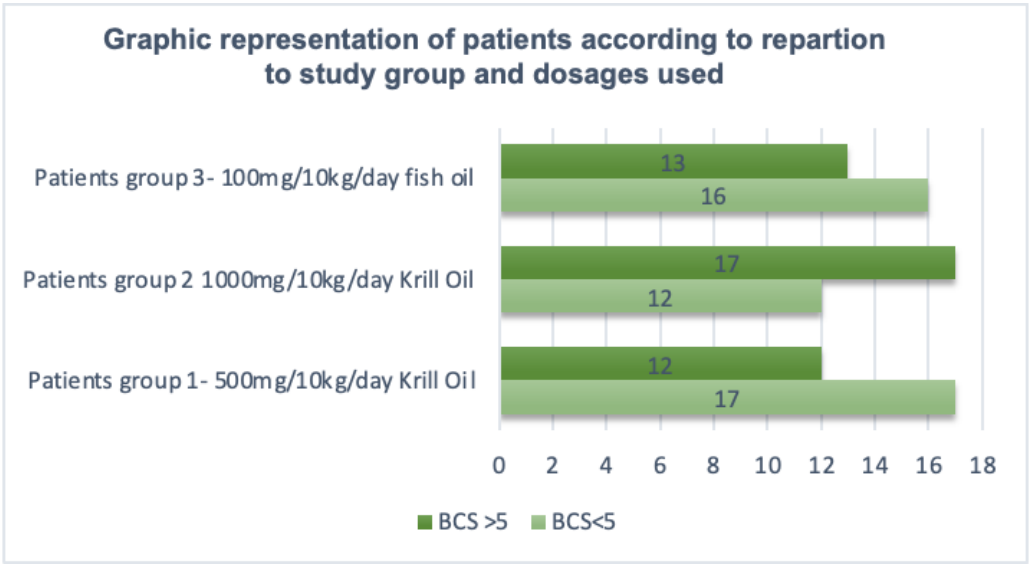

Fig. 5. Graphic representation of patients according to repartition in study group based on body condition score and dosages administered

Paraclinical biochemistry results concerning the values of cholesterol and triglycerides were analyzed using a statistical analysis called student test after a period of administration of Krill Oil or salmon oil for a period of 80 days.

Before conducting this test, we theorize that:

- The null hypothesis is that there is no significant difference between the median cholesterol and triglycerides levels and the standard median before and after administering the treatment
- The alternative hypothesis is that there is a significant difference between the median value of cholesterol and triglycerides and the standard median.

The condition in which this test can be conducted is if the σ2 variation is unknown, and the test applied to less than 30 subjects in a group. If these conditions are not met, the test loses its value. For this study, the authors of this paper have chosen an α (alpha) value of 5%, correlated with an acuity level of 95%.

After approximately 11 weeks of treatment, patients in group 1 which received 500 mg Krill Oil/day, with a body condition score less than 5 and respectively, 1000 mg Krill Oil/day, with a body condition score more than 5, experienced a decrease of 7.50%, respectively 8.26% in cholesterol values from median values of 418.812mg/dl to 387.375 mg/dl, with P values < 0.001, as shown in Table 3.

Median values of triglycerides decreased with 17.59% (for patients with BCS<5) and with 20.913% (for patients with BCS>5), taking into account P values less than 0.001 (Table 4) .Results found in patients in group 1 show a strong correlation between a small P value, less than 0.001, less than α (0.05) and a T value greater than 2 (2.1314-2.1603). The null hypothesis can be rejected and the alternative hypothesis accepted, meaning that the administration of Krill oil has produced a significant improvement in cholesterol and triglycerides values in canine patients.

Table 3

Table showing evolution in cholesterol and triglycerides levels in patients with BCS<5, receiving 500mg/10kg/day Krill Oil, after a period of administration of 80 days, percentual variation, P and T value

Krill Oil 500mg/10kg/zi BCS<5	Day 1 mg/dl	Day 80 mg/dl	% Variation	P Value	T Value
Cholesterol	**418.81**	**387.37**	**-7.506%**	**0.000006877**	**2.131**
Triglyceride	**265.75**	**219**	**-17.592**	**0.000501208**	**2.131**

Table 4

Table showing evolution in cholesterol and triglycerides levels in patients with BCS>5, receiving 1000mg/10kg/day Krill Oil, after a period of administration of 80 days, percentual variation, P and T value

Krill Oil 1000mg/10kg/zi BCS≥5	Day 1 mg/dl	Day 80 mg/dl	%Variation	P Value	T Value
Cholesterol	**363.78**	**333.71**	**-8.266%**	**0.000008803**	**2.160**
Trygliceride	**142.42**	**112.64**	**-20.913%**	**0.00001948**	**2.160**

By increasing the dosage of Krill Oil in patients in group 2 to 1 gram/day in patients with BCS<5 and 2 grams/day in patients with BCS>5, cholesterol values decreased with 7.84% (for those with BCS<5), respectively 6.66% (for those with BCS>5), when P values were less than 0.001.

Median value of triglycerides decreased as well with 18.82%, respectively 16.19%, with P values less than 0.001 (P=0.0025). Results show a strong correlation between P values less than 0.001 and T values bigger than 2 (in the 2.2009-2.1098

range). As a conclusion, the null hypothesis can be rejected and the alternative one accepted meaning that ingesting Krill oil over a long period of time can lead to a significant decrease in cholesterol and triglycerides levels in canine patients. Results can be seen in Table 5 and Table 6.

Table 5

Table showing evolution in cholesterol and triglycerides level in patients with BCS<5, receiving 1000mg/10kg/day Krill Oil, after a period of administration of 80 days, percentual variation, P and T value

Krill Oil 1000mg/10kg/zi BCS<5	Day 1 mg/dl	Day 80 mg/dl	%Variation	P Value	T Value
Cholesterol	312.41	287.91	-7.842%	0.000003378	2.200
Triglyceride	98.83	76.16	-18.828%	0.00000596	2.200

Table 6

Table showing evolution in cholesterol and tryglicerides level in patients with BCS>5, receiving 2000mg/10kg/day Krill Oil, after a period of administration of 80 days, percentual variation, P and T value

Krill Oil 2000mg/10kg/day BCS>5	Day 1 mg/dl	Day 2 mg/dl	%Variation	P Value	T Value
Cholesterol	357.38	333.72	-6.622%	0.000000097	2.109
Triglyceride	131.05	109.83	-16.193%	0.002543843	2.109

Subjects in group 3 experienced a variation in medication. They were administered Salmon oil, 15 ml/day/10kg in patients with a body condition score less than 5 and 30 ml/day/10kg in patients with a body condition score greater than 5. In patients with BCS<5, this lead to a decrease of cholesterol levels of 9.73% and a decrease of triglycerides levels of 7.91%. In both cases, P levels were less than 0.001, thus rejecting the null hypothesis and accepting the alternative one. In patience with BCS>5, cholesterol levels decreased with 3.78% and triglycerides levels with 2, 90%. P values were equal to 0.054, equal to α (0.05) (Fig. 6), thus showing that salmon oil, administered in a dosage of 30ml/10kg/day does not improve cholesterol and triglycerides levels. Results can be seen in Tables 7 and 8 and summarized in Fig. 7 and 8.

Table 7

Table showing evolution in cholesterol and triglycerides level in patients with BCS<5, receiving 15mg/10kg/day Salmon Oil, after a period of administration of 80 days, percentual variation, P and T value

Fish oil 15mg/10kg/day BCS<5	Day 1 mg/dl	Day 80 mg/dl	%variation	P Value	T Value
Cholesterol	**223.67**	**201.90**	**-9.730%**	**0.000033937**	**2.131**
Triglyceride	**129.56**	**119.31**	**-7.911%**	**0.003017384**	**2.131**

Table 8

Table showing evolution in cholesterol and tryglicerides level in patients with BCS>5, receiving 30ml/10kg/day Salmon Oil, after a period of administration of 80 days, procentual variation, P and T value

Fish Oil 30mg/10kg/zi BCS>5	Day 1 mg/dl	Day 80 mg/dl	%variation	P Value	T Value
Cholesterol	412.92	397.28	-8.788	0.000017571	2.160
Triglyceride	211.28	205.14	-2.907	0.054068799	2.150

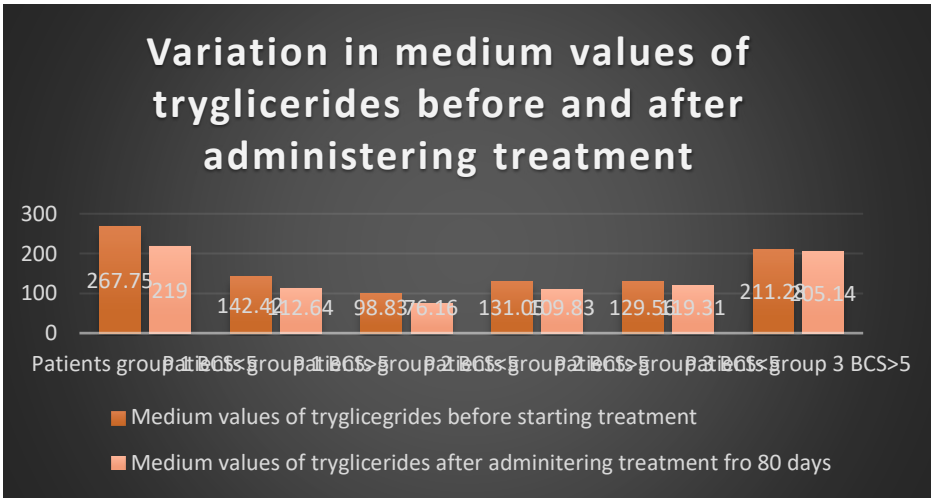

Fig. 7. Graph showing decrease in cholesterol levels after 80 days of treatment

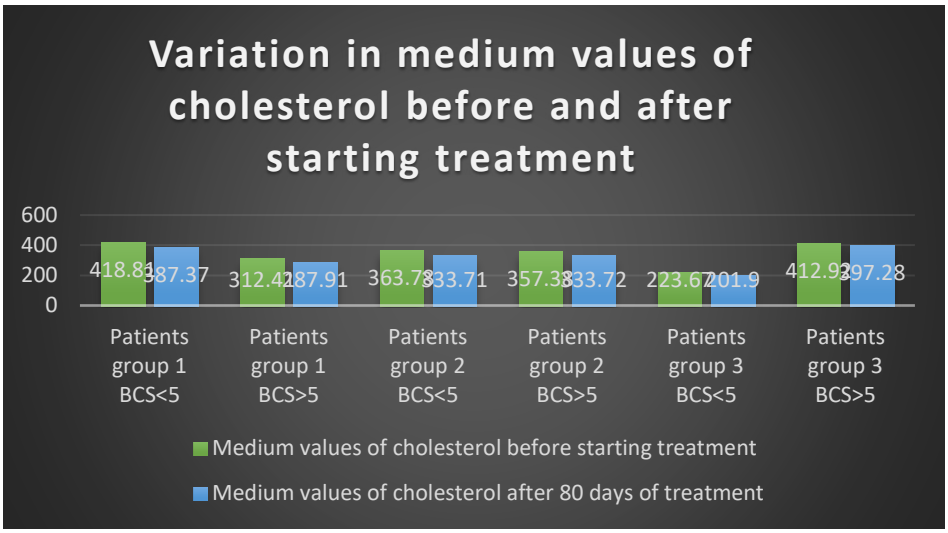

Fig. 8. Graph showing decrease in triglycerides levels after 80 days of treatment

Conclusions

Administering Krill and Salmon Oil in therapeutic doses has significantly reduced cholesterol and triglycerides levels in 83.33% (n=5) of the 6 groups of patients being analyzed.

Doubling the dose administered does not yield better results as seen in group 2, where patients with a body condition score greater than 5 experienced a decrease of 1.6% in cholesterol levels, compared to patients in group one with a body condition score greater than 5.

The same was noted in the evolution of triglycerides levels, where patients receiving 2 grams/ 10kg/day from the second group with a body condition score greater than 5 had a lower improvement rate of 16.193% compared to the ones from the first group with a body condition score greater than 5, who received 1 gram/10kg/day and experienced an improvement of 20.913% in triglycerides levels.

According to the research conducted by the authors of this paper, Krill oil proved to be superior in lowering cholesterol and triglycerides levels, but both Krill Oil and Salmon oil were efficient in significantly influencing cholesterol and triglycerides levels in canine patients suffering from hyperlipemia.

Acknowledgement

I would like to thank professor Mario Codreanu for supervision and guidance and doctor Alexandra Mihaela Popa for support.

References

1. **Appel, L.J., Miller 3rd, E.R., Seidler, A.J., Whelton, P.K.,** Does supplementation of diet with 'fish oil' reduce blood pressure? A meta-analysis of controlled trials, Archive of Internal Medicine, 1993, 153, 12, 1429-1438.
2. **Blok, W.L., Katan, M.B., Van der Meer, J.W.,** Modulation of inflammation and cytokine production by dietary (n-3) fatty acids, Journal of Nutrition, 1996, 126, 6, 1515-33.
3. **Bunea, R., El Farrah, K., Deutsch, L.,** Evaluation of the effects of Neptune Krill Oil on the clinical course of hyperlipidemia, Alternative Medicine Review, 2004, 9, 4, 420-428.
4. **Cansell, M., Moussaoui, N., Denizot, A., Combe, N.,** Influence of the physicochemical form of polyunsaturated fatty acids on their in vivo bioavailability; 94th Annual AOCS Meeting & Expo PHO1: Phospholipids for Improving Bioavailability Chair: Michael Schneider, Consulant, Germany, 2003.
5. **Codreanu, M., Orăşanu A., Constantinescu, I., Codreanu I., Rădoi I.,** Aspecte clinice, hematologice, biochimice şi ecografice la câinii cu afecţiuni hepatobiliare, Al IV-lea Simpozion Aniversar al Institutului de Diagnostic şi Sănătate Animală „35 de ani de activitate în diagnosticul medical veterinar pentru apărarea sănătăţii animale şi umane", Bucureşti, România, 2001.
6. **Codreanu, M.D., Crivineanu M.,** Terapeutică Veterinară, Editura Printech, 2008.
7. **Ettinger, S., Feldman, E.C.,** Textbook of Veterinary Internal Medicine Consult, Ed. Elsevier- Health Sciences Division, Amsterdam, 2016.
8. **Kahn, C.M., Line, S.,** Manualul Merck de Medicină Veterinară, ediția a X-a, Editura Callisto, București, 2014.
9. **McNamara, R.K., Carlson, S.E.,** Role of Omega-3 Fatty Acids in Brain Development and Function: Potential Implications for the Pathogenesis and Prevention of Psychopathology, Prostaglandins Leukotrienes Essential Fatty Acids, 2006, 75, 4-5, 329-49.
10. **Mihai, D., Codreanu, M.,** Medicina internă a animalelor, vol.III, Ed. Printech, Bucureşti, 2002.
11. **Nicol, S., Endo, Y.,** Krill fisheries of the world, FAO fisheries technical paper 367. Rome: FAO, 1997.
12. **Reiffel, J.A., McDonald, A.,** Antiarrhythmic effects of omega-3 fatty acids, American Journal of Cardiology, 2006, 98, 4A, 50i-60i.
13. **Reijnders, L., Soret, S.,** Quantification of the environmental impact of different dietary protein choices, American Journal of Clinical Nutrition, 2003, 78, 3, 6645-6685.
14. **Sampalis, F., Pelland, M., Duguet, N.,** Evaluation of the effect of Neptune Krill Oil on the management of Premenstrual Syndrome, Alternative Medicine Review, 2003, 8, 2, 171-179.
15. **Smelt, A.,** Triglycerides and gallstone formation, Clinica Chimica Acta, 2010,

411, 21-22, 1625-1631.
16. **Swell, L., Bell, C.B.Jr.,** Current Concepts of the Pathogenesis of Cholesterol Gallstones, Medical Clinics of North America, 1974, 58, 6, 1449-1471.
17. **Tandy, S., Chung, R., Wat, E., Kamili, A., Berge, K., Griinari, M., Cohn, J.,** Dietary krill oil supplementation reduced hepatic steatosis, gylcemia, and hypercholesterolemia in high-fat-fed mice, Journal of Agricultural and Food Chemistry, 2009, 57, 9339-9345.
18. **Tousoulis, D., Plastiras, A., Siasos, G., Oikonomou, E., Vervenitos, A., Kokkou, E., Maniatis, K., Goulipoulos, N., Miliou, A., Paraskevopoulos, T., Stefanadis, C.,** Omega-3 PUFAs improved endothelial function and arterial stiffness with a parallel antiinflammatory effect in adults with metabolic syndrome, Atherosclerosis, 2014, 232, 1, 10-16.
19. **Werner, A., Havinga, R., Kuipers, F., Verkade, H.J.,** Treatment of EFA deficiency with dietary triglycerides or phospholipids in a murine model of extrahepatic cholestasis, American Journal of Physiology, Gastrointestinal and liver physiology, 2004, 286, 5, 822-32.
20. **Zhu, J.J., Shi, J.H., Qian, W.B., Cai, Z.Z., Li, D.,** Effects of Krill Oil on Serum Lipids of Hyperlipidemic Rats and Human SW480 Cells, Lipids in Health and Disease, 2008, 7, 30.

THE AGE INFLUENCE ON THE INCIDENCE OF FELINE CHRONIC KIDNEY DISEASE

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Summary

The prevalence of chronic kidney disease in cats increased significantly in recent years, requiring the identification of the etiopathogenetic context in correlation with the systemic effects and functional organ disorders, for the application of a diagnostic algorithm and the optimization of the curative protocols. The study was conducted over a period of two years, within the University Emergency Hospital Prof. Univ. Dr. Alin Bîrțoiu, Bucharest on a number of 20 cases of felines with suspicion of chronic kidney disease, 10 of these, ranged in age from 5 to 18 years, being considered eloquent for our study. The clinical examination was performed on each patient separately, by general methods, as well as by special, paraclinical methods, such as: biochemical and hematological examination, urine sediment examination, ultrasound examination and in some cases, cardiological exam. The results obtained from the paraclinical examinations and the physiological limits were specified in each individual case. Regarding the age of the patients that had a positive diagnosis of chronic kidney disease, the oldest age was recorded in the sixth case (18 years), in the third, fourth and last case, the age of the patients was 15 years. There were 2 patients aged 12 years (cases eight and nine) and a single patient aged 10 years (the second).There were 3 patients under the age of 10, namely: the first case, the fifth and the seventh. Of the patients with chronic kidney disease included in the present study (n=10) belonging to the European race (100%), 6 females (n=60%), 4 males (n=40%), 7 individuals (70%) belonged to the age group > 10 years, and the rest (n=3), under this age. Therefore, 70% of the diagnosed cases were recorded in geriatric patients with ages ranging from 10 to 18 years, only 30% of the cases being recorded in younger (less than 10 years old) patients.

Keywords: felines, chronic kidney disease, geriatric patients.

The most frequent renal disease in felines is known as chronic kidney disease (CKD), which is characterized by structural and/or functional impairment in one or both kidneys that has lasted longer than three months (1, 2, 3). CKD can be the result of any condition that injures the kidneys in a sense that is progressive and irreversible and it is actually intimately associated to a variety of diseases that develop after kidney damage begins (3, 4, 20). A multitude of variables, including aging, ischemia, comorbid conditions, phosphorus overload, and routine vaccinations, have been associated to the occurrence of this disease in affected cats (6, 7). Dietary phosphorus intake, the magnitude of proteinuria and anemia are all associated with the progression of established CKD, which occurs in some but not all cats (8, 19).

Age is one of the most important risk factors in chronic kidney disease of domestic felines (5, 9, 13). These can develop chronic kidney disease at any age. Juvenile onset chronic kidney disease is most likely the result of a family history of renal disease (4, 6, 7). With these exceptions, this pathology is more common in older felines. However, older felines may also have other age-related disease processes that contribute to the development of chronic kidney disease (11). Regardless of age, cats have an incidence of 1-3% of the total feline population and as feline age, the percentage of affected patients rises to 30%, with an average age of around 7.4 years (10,12, 17).

Together with paraclinical examinations, which have recently become more efficient and accurate in establishing the diagnosis of chronic kidney disease, the clinical exam had and continues to have great importance in establishing the diagnosis and consequently, the treatment (14, 15, 16, 18).

Materials and methods

The present experiment was carried out in the University Emergency Hospital Prof. Univ. Dr. Alin Bîrțoiu, Bucharest.

In the present experiment it was formed and studied a group of 10 felines, age ranged from 5 to 18 years during a period of 2 years (March 2020 – April 2022).

The main selection criteria were represented by: felines of various ages of the European breed which was fed with food containing good quality protein as well as food containing poor quality protein.

The investigate ensemble was hematological investigations (complete blood count), blood biochemistry (urea, creatinine, total serum proteins, albumin, phosphorus, sodium and serum potassium) in all 10 patients; blood samples for biochemical and hematological examination; ultrasound examination.

Results and discussions

The blood biochemistry tests results for each of the ten subjects in the studied group of felines are presented in table 1.

All patients present at the study have urea values above the reference range (27.8-79.2 mg/dL), the same aspect being recorded in the case of creatinine (0.7-2.0 mg/dL). Only two patients had values that were higher than the reference interval (3.5-5.8 mmol/L) for potassium and six felines had values that were higher than the reference interval for phosphorus (3.1-7.5 mg/dL), which was determined to seven out of ten patients.

All patients consumed food containing low quality protein, and none of the patients in the study consumed the renal diet before presenting to the hospital and performing the biochemical examination.

Table 1

The results of the felines biochemical examination upon the arrival at the hospital

Pacient	CREA	BUN	K	P
Athena	**8.26** (mg/dL)	**208** (mg/dL)	5.4 (mmol/L)	**7.57** (mg/dL)
Peony	**7.2** (mg/dL)	**195** (mg/dL)	-	-
Felix	**3.45** (mg/dL)	**191** (mg/dL)	5.35 (mmol/L)	**7.35** (mg/dL)
Fluffy	**3.64** (mg/dL)	**134** (mg/dL)	5.0 (mmol/L)	4.76 (mg/dL)
Josephine	**15** (mg/dL)	**380** (mg/dL)	**8** (mmol/L)	**7.2** (mg/dL)
Kitty	**5.5** (mg/dL)	**282** (mg/dL)	3.5 (mmol/L)	**9.7** (mg/dL)
Cinnamon	**19.01** (mg/dL)	**637** (mg/dL)	-	**27.21** (mg/dL)
Pinky	**10.2** (mg/dL)	**272** (mg/dL)	**6.7** (mmol/L)	**>18** (mg/dL)
Pumpkin	**5.5** (mg/dL)	**188** (mg/dL)	-	-
Tiger	**6.3** (mg/dL)	**197** (mg/dL)	-	-

The food can be one of the determining factors of chronic kidney disease in felines, particularly in terms of protein quality and sodium and potassium content.

Regarding the patients' ages, the adjacent figure (Fig. 1) shows that the sixth case (18 years) had the oldest age recorded, while the third, fourth, and last cases all had patients who were 15 years old. They have two patients (the eighth and ninth cases) who are 12 years old and one patient who is 10 years old on record (the second). Three of the patients—the first case, the fifth patient, and the seventh patient—were under the age of 10.

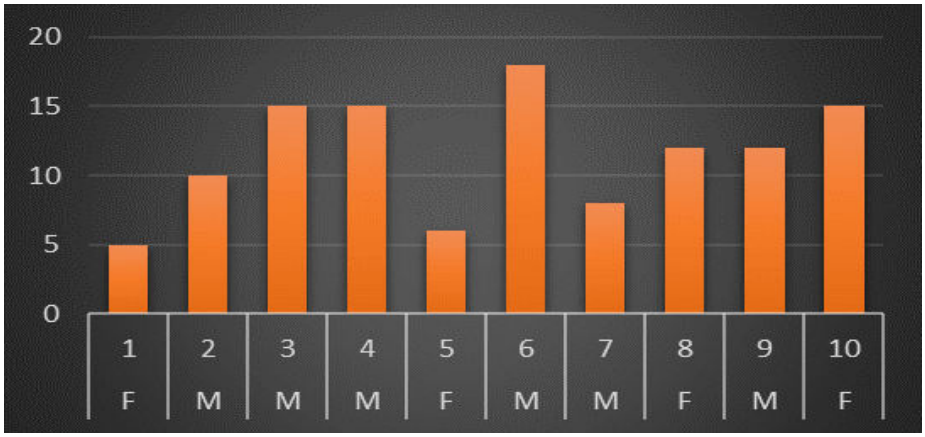

Fig. 1. Graphic representation of the patients' age
Left: age of patients; horizontally: No of patient and sex

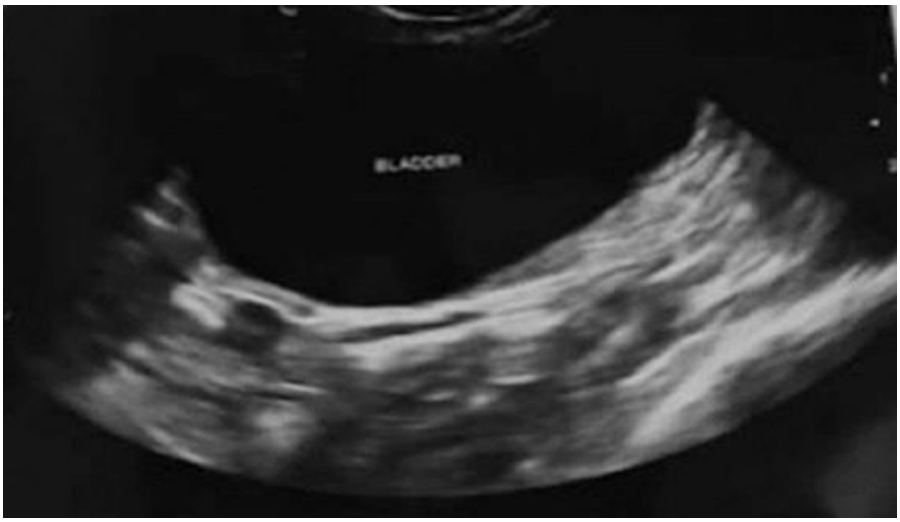

Fig. 2. Ultrasonographic aspect of the urinary bladder

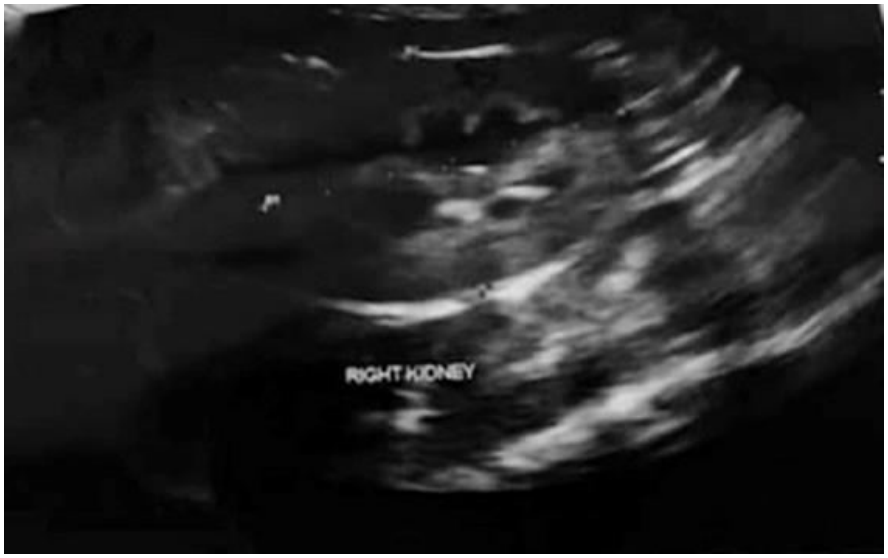

Fig. 3. Ultrasonographic aspect of the right kidney

The renal cortex appears to be dysregulated structurally, as well as in terms of shape and hyperechogenic, while the specific structure of the medulla is altered, as you can see in (Fig. 3), specific aspect for chronic kidney disease. (Kitty's ultrasonography images). In (Fig. 2), it's represented the urinary bladder filled with urine, for the same patient.

Conclusions

Hematological tests (blood count), blood biochemicals (urea, creatinine, urea nitrogen, protein total serum, albumin, serum phosphorus, sodium, and potassium), and clinical examination were all part of the investigational ensemble used to detect, confirm, and stage chronic renal disease. The clinical dominants in patients with advanced stages of CKD/CKD were represented by the tendency to hypothermia, anorexia, dehydration, vomiting, neurological depression, of different degrees and intensities.

Serum urea and creatinine levels served as the diagnostic components that determined and verified the level of nitrogen retention (in all patients), requiring the performance of additional tests with functional relevance and serving as the initial therapeutic goal.

The oldest case involved a patient who was 18 years old, while the third, fourth, and final cases involved patients who were 15 years old.

Patients with the highest creatinine and urea levels (CREA - 19.01 mg/dL; UREE - 637 mg/dL) were 8-year-old, while those with the lowest levels (3.5 mg/dL, 191 mg/dL; 3.6 mg/dL, 134 mg/dL) were all 15-year-old.

Seven of the total number of patients included in this study (n=7) belonged to the age group 10 + years, the remaining (n=3) being under this age.

Acknowledgement

The research was carried out as part of an extensive study, the preliminary results being part of the Bachelor's Thesis *"Research on the therapeutic approach and the incidence of chronic kidney disease in felines".*

References

1. **Blackwell, W.,** Feline internal medicine, Elsevier, Health Sciences Division, 2013.
2. **Brown, C.A., Elliott, J., Schmiedt, C.W., Brown, S.A.,** Chronic Kidney Disease in Aged Cats: Clinical Features, Morphology, and Proposed Pathogeneses, Veterinary Pathology, 2016, 53, 2, 309-326.
3. **Chew, D.J., Dibartola, S.P., Schenck, P.A.,** Canine and Feline nephrology and urology, BSAVA, 2011.
4. **Da, J., Xie, X., Wolf, M., Disthabanchong, S., Wang, J., Zha, Y., Lv, J., Zhang, L., Wang, H.,** Serum Phosphorus and Progression of CKD and Mortality: A Meta-analysis of Cohort Studies, American Journal of Kidney Diseases, 2015, 66, 2, 258-265.
5. **Dobenecker, B., Webel, A., Reese, S., Kienzle, E.,** Effect of a high phosphorus diet on indicators of renal health in cats, Journal of Feline Medicine and Surgery, 2018, 20, 4, 339-343.
6. **Finch, N.,** Measurement of glomerular filtration rate in cats: methods and advantages over routine markers of renal function, Journal of Feline Medicine and Surgery, 2014, 16, 9, 736-748.
7. **Finch, N.C., Syme, H.M., Elliott, J.,** Risk Factors for Development of Chronic Kidney Disease in Cats, Journal of Veterinary Internal Medicine, 2016, 30, 2, 602-610.
8. **Greene, J.P., Lefebvre, S.L., Wang, M., Yang, M., Lund, E.M., Polzin, D.J.,** Risk factors associated with the development of chronic kidney disease in cats evaluated at primary care veterinary hospitals, Journal of the American Veterinary Medical Association, 2014, 244, 3, 320-327.
9. **Hill, P., Shawcross, G., Warman, S.,** 100 top consultations in small animals general practice, Wiley-Blackwell Publishing, 2011.
10. **Kruth, S.,** Textbook of Veterinary Internal Medicine, Elsevier Saunders, 6th edition, 2006.

11. **Loane, S.C., Thomson, J.M., Williams, T.L., McCallum, K.E.,** Evaluation of symmetric dimethylarginine in cats with acute kidney injury and chronic kidney disease, Journal of Veterinary Internal Medicine, 2022, 36, 5, 1669-1676.
12. **Piyarungsri, K., Pusoonthornthum, R.,** Risk and protective factors for cats with naturally occurring chronic kidney disease, Journal of Feline Medicine and Surgery, 2017, 19, 4, 358-363.
13. **Pressler, B.M.,** Clinical approach to advanced renal function testing in dogs and cats, The Veterinary clinics of North America, Small animal practice, 2013, 43, 6, 1193.
14. **Reynolds, B.S., Lefebvre, H.P.,** Feline CKD: Pathophysiology and risk factors--what do we know?, Journal of Feline Medicine and Surgery, 2013**,** 15,1, 3-14.
15. **Schauf, S., Coltherd, J.C., Atwal, J., Gilham, M., Carvell-Miller, L.J., Renfrew, H., Elliott, J., Elliott, D., Bijsmans, E.S., Biourge, V.C., Watson, P., Bakke, A.M.,** Clinical progression of cats with early-stage chronic kidney disease fed diets with varying protein and phosphorus contents and calcium to phosphorus ratios, Journal of Veterinary Internal Medicine, 2021, 35, 6, 2797-2811.
16. **Schauf, S., Coltherd, J.C., Atwal, J., Gilham, M., Carvell-Miller, L.J., Renfrew, H., Elliott, J., Elliott, D., Bijsmans, E.S., Biourge, V.C., Watson, P., Bakke, A.M.,** Clinical progression of cats with early-stage chronic kidney disease fed diets with varying protein and phosphorus contents and calcium to phosphorus ratios, Journal of Veterinary Internal Medicine, 2021, 35, 6, 2797-2811.
17. **Syme, H.M., Elliott, J.,** Risk factors for Development of Chronic Kidney Disease in Cats, Journal of Veterinary Internal Medicine, 2016, 30, 2, 602-610.
18. **Thanaboonnipat, C., Sutayatram, S., Buranakarl, C., Choisunirachon, N.,** Renal ultrasonographic strain elastography and symmetric dimethylarginine (SDMA) in canine and feline chronic kidney disease, Journal of Veterinary Medical Science, 2020, 82, 8, 1104-1112.
19. *** http://www.iris-kidney.com/education/education/risk_factors.html - IRIS - International Renal Interest Society
20. ***https://www.idexx.com/media/filer_public/7f/08/7f0886ce-2f03-460a-bf0a-cec1dd47070b/iris-pocket-guide-2019.pdf

THE USE OF THE SYMMETRIC DIMETHYLARGININE (SDMA) SCREENING IN THE DIAGNOSIS OF FELINE CHRONIC KIDNEY DISEASE

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Summary

The renal biomarker symmetric dimethylarginine (SDMA) is currently considered by specialty literature as the only early indicator in chronic kidney disease. SDMA highlights the alteration of the glomerular filtration rate's normal functioning, from the point when the nephrons have been damaged and destroyed in a 25% percentage. SDMA is an important biomarker in the early detection of kidney disease and can be interpreted in correlation with the serum creatinine level, but also evaluated alone, symmetric dimethylarginine being much more sensitive in early detecting chronic kidney disease than creatinine. Our study was performed on a group of 10 feline patients with different stages of chronic kidney disease, studied over a period of two years at University Emergency Hospital Prof. univ. Dr. Alin Bîrțoiu, Bucharest. The investigative ensemble used for identifying, confirming, and evaluating the stage of the chronic kidney disease, in addition to the clinical examination included hematological investigations (complete blood count), blood biochemistry (urea blood nitrogen, creatinine, total serum proteins, albumin, phosphorus, sodium and serum potassium) in all 10 patients and symmetrical dimethylarginine (SDMA) in 5 patients (50%). In order to confirm the diagnosis ultrasound examination was also performed in all patients. With maximum accuracy in establishing (according to the IRIS staging) the degree of functional impairment, the use of the SDMA test performed in 50% of the patients allowed us to confirm the diagnosis and to establish the optimal therapeutic protocol, in the case of the patients with SDMA values over 20 micrograms/dL.

Keywords: symmetric dimethylarginine, SDMA, felines, chronic kidney disease.

A precise identification of the etiopathogenetic context in addition to systemic and organ-specific effects is necessary in order to apply a diagnostic algorithm and to optimize the treatment protocols because the prevalence of feline chronic kidney disease has significantly increased in recent years (1, 5, 6). Specialty literature currently considers the renal biomarker symmetric dimethylarginine (SDMA) as the only early sign of chronic kidney disease (2, 3, 4). Since symmetric dimethylarginine is much more sensitive than creatinine at detecting chronic kidney disease early on, it is a key biomarker in the early detection of kidney disease (4, 8). SDMA can be evaluated alone or in correlation with the serum creatinine level (3, 19).

The symmetric dimethylarginine is produced by the intranuclear methylation of L-arginine by arginine-methyltransferase, which is then released into the blood following proteolysis (2, 16, 18). It closely correlates with glomerular filtration, which

is its primary method of elimination, and is unaffected by tubular reabsorption or secretion (5, 8, 9). Additionally, in human medicine, symmetric dimethylarginine (SDMA) is a crucial biomarker for the early diagnosis of kidney disease and can be interpreted in relation to serum creatinine (9, 11, 12). However, when it's evaluated alone, symmetric dimethylarginine is much more sensitive than creatinine at identifying chronic kidney disease (10, 11, 17).

Creatinine values of 140 mol/l and 1.6 mg/dl, and SDMA concentrations of 18 g/dl are indicative of the first stage of chronic kidney disease in felines patients (6, 7). These values indicate that creatinine is within normal ranges and that SDMA concentration is only slightly elevated (13). Early detection of chronic kidney disease is possible if this rise in the glomerular filtration rate parameter concentration lasts longer than 14 g/dl. In the second stage, higher levels of creatinine (140–250 mol/l and 1.6–2.8 g/dl) and SDMA (26–38 g/dl) are present and a mild renal azotemia develops (1, 2). Clinical symptoms are either barely perceptible or nonexistent during this stage. Clinical symptoms start to appear in stage 3 but their severity varies from patient to patient (7, 14).

Clinical symptoms begin to appear in stage 3 but their severity varies from patient to patient. This stage can be recognised as early if there are no symptoms, and it will be characterized as late stage 3 if there are obvious manifestations (20). At this point, the levels of creatinine are 251-440 mol/l, 2.9–5 g/dl, and 26–38 for SDMA. The most severe and final stage of chronic kidney disease, stage 4, is marked by severe systemic clinical manifestations, the development of a uremic coma, creatinine values greater than 440 mol/l and 5 g/dl, and SDMA greater than 39. The prognosis is grim (1, 3, 15).

Materials and methods

The present experiment was carried out in the University Emergency Hospital Prof. univ. Dr. Alin Bîrțoiu, Bucharest.

In the current study, a group of 10 felines with ages ranging from 5 to 18 was organized and noticed over the course of two years (March 2020 – April 2022).

Felines of different ages of the European breed, fed both food with high-quality protein and food with low-quality protein, represented the main selection criteria.

Hematological examinations (complete blood counts), blood biochemistry (urea blood nitrogen, creatinine, total serum proteins, albumin, phosphorus, sodium, and serum potassium) tests, blood samples for hematological and biochemical analysis, and the SDMA test to measure the severity of glomerular filtration injury constituted the investigational group (incipient or advanced).

Results and discussions

The blood biochemistry tests results for patients with SDMA test in the studied group of felines are presented in table 1.

Table 1

Biochemical results for the patients with SDMA test

Patient	CREA	BUN	K	P
Athena	**8.26** (mg/dL)	**208** (mg/dL)	**5.4** (mmol/L)	**7.57** (mg/dL)
Felix	**3.45** (mg/dL)	**191** (mg/dL)	**5.35** (mmol/L)	**7.35** (mg/dL)
Fluffy	**3.64** (mg/dL)	**134** (mg/dL)	**5.0** (mmol/L)	**4.76** (mg/dL)
Cinnamon	**19.01** (mg/dL)	**637** (mg/dL)	**-**	**27.21 (mg/dL)**
Tiger	**6.3** (mg/dL)	**197** (mg/dL)	**-**	**-**

The BUN (urea blood nitrogen) values of all the study participants are above the reference range (27.8-79.2 mg/dL) and the creatinine values are also above the reference range (0.7-2.0 mg/dL). Only three patients had phosphorus values that were higher than the reference interval (3.1-7.5 mg/dL). One patient from the study group was the only one who did not notice increasing potassium and phosphorus levels.

Table 2

SDMA results

Pacient		SDMA
Athena		**23 µg/dL**
Felix		**23µg/dL**
Fluffy		**20 µg/dL**
Cinnamon		**26 µg/dL !!!**
Tiger		**22 µg/dL**

Symmetric dimethylarginine (SDMA) usually has values that are less than or equal to 15 µg/dL. Each patient who took part in the research had values higher than 15 µg/dL. The patients who participated in the study had a minimum value of 26 µg/dL and the minimum was 20 µg/dL.

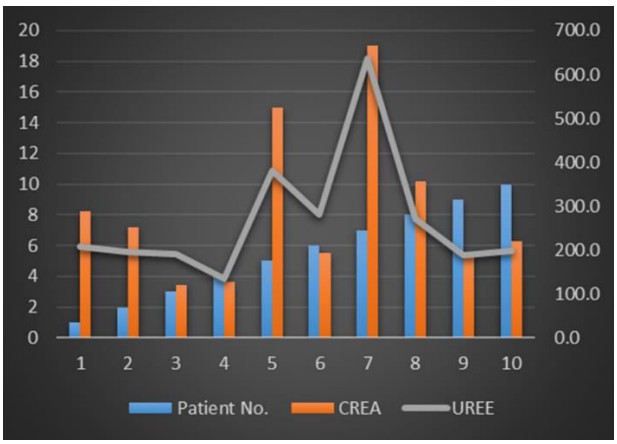

Fig. 1. Biochemical results for all 10 patients

The results of the creatinine and BUN tests are presented in the Fig. 1. The patient in the seventh case had the highest values (CREA: 19.01 mg/dL; BUN: 637 mg/dL), and the patient in the fifth case had the lowest values (CREA: 15 mg/dL; BUN: 380 mg/dL). The third and fourth cases had the lowest urea and creatinine test results (3.5 mg/dL, 191 mg/dL; 3.6 mg/dL, 134 mg/dL).

Conclusions

Older felines with chronic kidney disease (CKD) are more likely to be diagnosed as they get older. Traditional indirect glomerular filtration rate (GFR) biomarkers have limitations and are ineffective at identifying glomerular filtration rate declines that occur early. Recent studies have suggested the use of symmetric dimethylarginine (SDMA) concentrations as a novel biomarker of GFR for the early diagnosis of CKD.

The investigational ensemble used to identify, confirm and stage chronic renal disease included hematological tests (blood count), blood biochemicals (urea, creatinine, urea nitrogen, protein total serum, albumin, serum phosphorus, sodium, and potassium) and clinical examination.

Using the SDMA test (performed on 5 patients) allowed for the most accurate determination of the optimal therapeutic protocol for feline chronic kidney disease (in the case of those with values higher than 20 micrograms/dL).

Patients who had the highest levels of creatinine (8.26 mg/dL; 9.01 mg/dL) and BUN (208 mg/dL; 637 mg/dL) had also had the highest SDMA test results (23µg/dL; 26 µg/dL).

Patients who had the highest SDMA test results (23µg/dL; 26 µg/dL); had also the highest levels of phosphorus (7.57mg/dL; 27.21 mg/dL).

Acknowledgement

The research was carried out as part of an extensive study, the preliminary results being part of the Bachelor's Thesis *"Research on the therapeutic approach and the incidence of chronic kidney disease in felines".*

References

1. **Baral, R.M., Freeman, K.P., Flatland, B.,** Analytical quality performance goals for symmetric dimethylarginine in cats, Veterinary clinical pathology, 2021, 50, 1, 57-61.
2. **Bilbrough, G., Evert, B., Hathaway, K., Panagakos, G., Robertson, J., Yerramilli, M.,** IDEXX Catalyst SDMA Test for in-house measurement of SDMA concentration in serum from dogs and cats, IDEXX Laboratories, 2018.
3. **Brans, M., Daminet, S., Mortier, F., Duchateau, L., Lefebvre, H.P., Paepe, D.,** Plasma symmetric dimethylarginine and creatinine concentrations and glomerular filtration rate in cats with normal and decreased renal function, Journal of Veterinary Internal Medicine, 2021, 35, 1, 303-311.
4. **Brown, C.A., Elliott, J., Schmiedt, C.W., Brown, S.A.,** Chronic Kidney Disease in Aged Cats Clinical Features, Morphology, and Proposed Pathogeneses, Veterinary Pathology, 2016, 53, 2, 309-326.
5. **Chew, D.J., Dibartola, S.P., Schenck, P.A.,** Canine and Feline nephrology and urology, Second Edition, BSAVA, Elsevier, The Ohio State University, Columbus, OH, 2011.
6. **Da, J., Xie, X., Wolf, M., Disthabanchong, S., Wang, J., Zha, Y., Lv, J., Zhang, L., Wang, H.,** Serum Phosphorus and Progression of CKD and Mortality: A Meta-analysis of Cohort Studies, American Journal of Kidney Diseases, 2015, 66, 2, 258-265.
7. **Finch, N.,** Measurement of glomerular filtration rate in cats: methods and advantages over routine markers of renal function, Journal of Feline Medicine and Surgery, 2014, 16, 9, 736-748.
8. **Finch, N.C., Syme, H.M., Elliott, J.,** Risk Factors for Development of Chronic Kidney Disease in Cats, Journal of Veterinary Internal Medicine, 2016, 30, 2, 602-610.
9. **Greene, J.P., Lefebvre, S.L., Wang, M., Yang, M., Lund, E. M., Polzin, D.J.,** Risk factors associated with the development of chronic kidney disease in cats

evaluated at primary care veterinary hospitals, Journal of the American Veterinary Medical Association, 2014, 244, 3, 320-327.
10. **Grelová, S., Karasová, M., Tóthová, C., Kisková, T., Baranová, D., Lukáč, B., Fialkovičová, M., Micháľová, A., Kunay, L., Svoboda, M.,** Relationship between FGF 23, SDMA, Urea, Creatinine and Phosphate in Relation to Feline Chronic Kidney Disease, Animals, 2022, 12, 17, 2247.
11. **Hall, J.A., Yerramilli, M., Obare, E., Yerramilli, M., Yu, S., Jewell, D.E.,** Comparison of serum concentrations of symmetric dimethylarginine and creatinine as kidney function biomarkers in healthy geriatric cats fed reduced protein foods enriched with fish oil, L-carnitine, and medium-chain triglycerides, Veterinary Journal, 2014, 202, 3, 588-96.
12. **Michael, H., Szlosek, D., Clements, C., Mack, R.,** Symmetrical Dimethylarginine: Evaluating Chronic Kidney Disease in the Era of Multiple Kidney Biomarkers, Veterinary Clinics of North America, Small Animal Practice, 2022, 52, 3, 609-629.
13. **Nabity, M.B.,** Traditional Renal Biomarkers and New Approaches to Diagnostics, Toxicologic Pathology, 2018, 46, 8, 999-1001.
14. **Piyarungsri, K., Pusoonthornthum, R.,** Risk and protective factors for cats with naturally occurring chronic kidney disease, Journal of Feline Medicine and Surgery, 2017, 19, 4, 358-363.
15. **Reece, W.O., Rowe, E.W.,** Functional Anatomy and Physiology of Domestic Animals, BSAVA, Iowa, SUA, 2017.
16. **Relford, R., Robertson, J., Clements, C.,** Symmetric Dimethylarginine: Improving the Diagnosis and Staging of Chronic Kidney Disease in Small Animals, Veterinary Clinics of North America, Small Animal Practice, 2016, 46, 6, 941-960.
17. **Syme, H.M., Elliott, J.,** Risk Factors for Development of Chronic Kidney Disease in Cats, Journal of Veterinary Internal Medicine, 2016, 30, 2, 602-610.
18. **Van den Broek, D.H., Chang, Y.M., Elliott, J., Jepson, R.E.,** Chronic Kidney Disease in Cats and the Risk of Total Hypercalcemia, Journal of Veterinary Internal Medicine, 2017, 31, 2, 465-475.
19. ***http://www.iris-kidney.com/education/education/risk_factors.html - IRIS - International Renal Interest Society
20. ***https://www.idexx.com/en/veterinary/reference-laboratories/sdma/interpreting-your-sdma-results/#imm

LUCRĂRI ŞTIINŢIFICE MEDICINĂ VETERINARĂ VOL. LVI(1), 2023, TIMIŞOARA

STUDY ON THE CLINICAL-THERAPEUTIC MANAGEMENT OF ANEMIAS IN COMPANION ANIMALS

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Summary

The medical literature data clearly highlights the rigor and the major importance of the clinical examination in establishing the diagnosis, but to the same extent considers that, in the current era, modern paraclinical methods of great precision and finesse, have considerably widened the field of investigation, offering a scientific model of interpretation of symptoms and diagnosis. The present study represents a concentrated update of bibliographic data regarding anemias in companion animals. The work comes as a response to the requirements of the veterinary medical practice, which is faced with a very diversified casuistry from the point of view of the etiopathological complex of anemia, which implies a differentiated clinical-therapeutic approach. Managing the cases included in the report, we followed a unitary investigative protocol, aimed at recording, drawing up clinical observation sheets, the general clinical examination, the functional and physical examination of the circulatory system (in correlation with the data of the other existing devices), hematological and biochemical investigations aiming especially the red line.

Keywords: anemia, pets, hematological.

In correlation with the multiplicity and intricate intervention of factors and the pathogenetic context and constituting the implications of the production of anemia in small animals, we considered it appropriate to approach a unitary clinical and investigative protocol, aiming at a thorough clinical evaluation in dynamics.

These elements intervene argumentatively in the opportunity of the correct and complex approach to cases of anemia, all the more so since most of them are considered medical emergencies.

Materials and methods

The study was carried over a period of two and a half years, on a number of 30 animals in The University Emergency Hospital “Alin Bîrțoiu” București. The patients were brought to the control by their owners due to clinical signs such as drowsiness, apathy, loss of appetite, discoloration of mucous membranes and changes in behavior. The animals in our study are described in Table 1.

The following work methods were used to examine the animals: The general clinical examination of the patient aims at obtaining the information collected in following both the inspection and the transabdominal palpation. The clinical examination also includes rectal thermometry, examination of apparent mucous membranes, identification of the patient's weight, respiratory rate, pulse, capillary

refill time (TRC), but also the degree of dehydration. The auscultation was carried out with the help of the stethoscope, following the recording of the heart and respiratory frequency.

Table 1

The structure of the study group diagnosed with anemia

Species	Breed	Sex	
		F	M
Dog	Mixed Breed	5	3
	Bichon	1	1
	Shih-Tzu	-	1
	German Shepherd	1	4
	Cocker Spaniel	1	-
	Pekingese	-	1
Cat	Mixed Breed	3	4
	Burmese	1	1
	Siamese	-	1
	British Short Hair	2	-

Transabdominal palpation was also performed, in all ten patients, bimanually, to highlight the presence or absence of deep pain sensitivity. Thermometry is also a general method of examination, consisting in the objective measurement of body temperature.

The functional examination was performed by collecting peripheral blood from the level of the saphenous vein, using a syringe with an 18 gauge needle. The blood thus collected was processed with the help of a hematological analysis device and blood smears were examined with the help of an optical microscope (Fig. 1).

The blood count was performed with the IDEXX VetAutoread Hematology Analyzer (Fig. 2) identifying the elements of the red cell line: RBC – number of red blood cells, HCT – hematocrit, HGB – hemoglobin, MCV – red blood cell volume average, MCH – globular average of of hemoglobin, MCHC – the average concentration of hemoglobin, the elements of the white line in terms of both quantity (the number of leukocytes) and quality (the percentage examination of the categories of leukocytes within the leukocyte formula), PLT – the number of platelets, MPV – the average platelet volume, RDW – erythrocyte size variation coefficient.

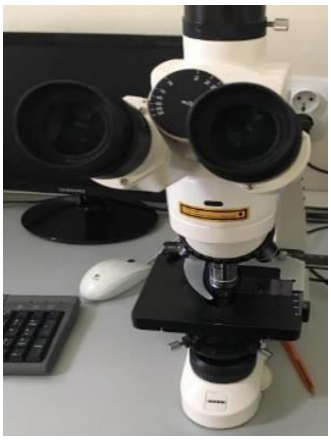

Fig. 1. Optic microscope Eurostar III plus

Fig. 2. IDEXX VetAutoread

Results and discussions

In the following table, the cases that represented the study of our paper will be described (Table 2).

Table 2

Structure of the cases presented in our study

Crt. No.	Diagnosis	Breed	No. of patients
1.	Immune mediated hemolytic anemia	Feline	1
		Canine	4
2.	Chronic renal insufficiency	Feline	7
		Canine	1
3.	Babesios	Feline	-
		Canine	8
4.	Poisoning with anticoagulants	Feline	-
		Canine	3
5.	Copper posioning	Feline	-
		Canine	1
6.	Giardiasis	Feline	-
		Canine	2
7.	Feline infectious peritonitis	Feline	3
		Canine	-

From a therapeutic point of view, the most severe cases, that represented medical emergencies, regarding very low values of the red blood line: red blood cells, hematocrit and hemoglobin, transfusion therapy with whole blood or erythrocyte concentrate was instituted. The treatment of anemia in all patients was represented by oxygen therapy and hematinic medicinal substances, intended to stimulate hematopoiesis, with the institution of transfusion therapy in the case of severe anemia. Of course, depending on the cause that determined the anemia, etiotropic therapy was also approached.

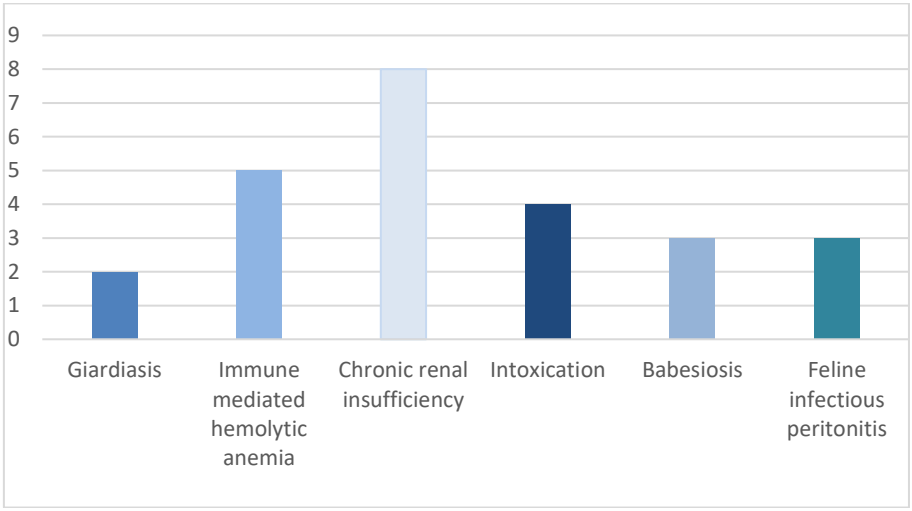

Fig. 3. Graphic representation of the cases included in our study

Figure 3 represents the graphic description of the cases included in the present study that determined anemia: 2 cases of giardiasis, 5 cases of immune-mediated hemolytic anemia, 8 cases of chronic renal failure, 4 cases of intoxication, 8 case of babesiosis and 3 cases of feline infectious peritonitis.

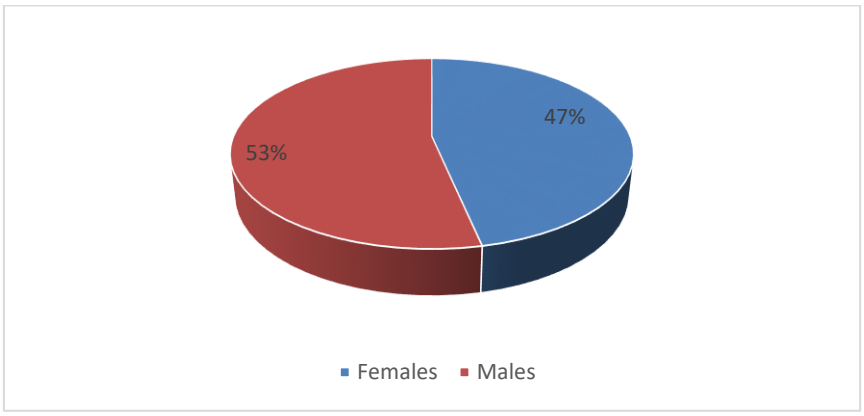

Fig. 4. Graphic representation of the sex predisposition of the animals included in our study

Our study contains 30 cases, of which, 14 females (46,6%), and 16 males (53,3%), as suggested in the second graph (Fig. 4), although in the literature there is no data according to which there is a predisposition of sex for the development of this circulatory pathology.

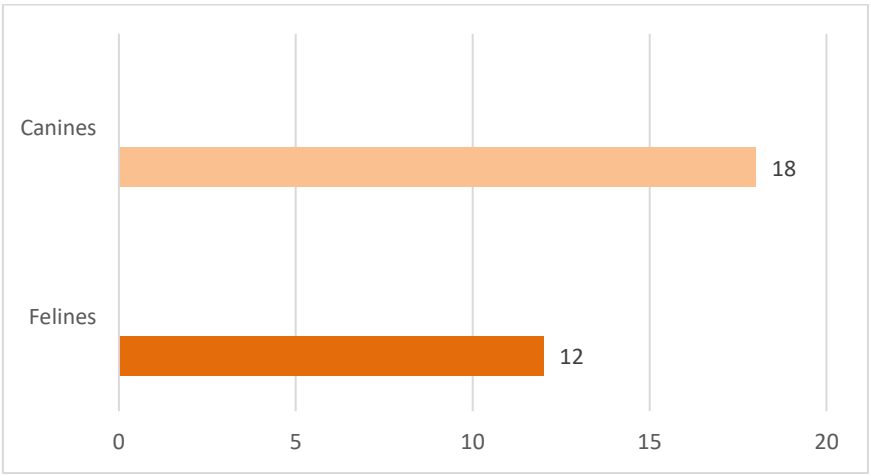

Fig. 5. Graphic representation of the species included in our study

Regarding the species that were included in the present study (Fig. 5), canids are represented in a percentage of 60% (n=18), while felines only in a

percentage of 40% (n=12), showing the higher prevalence of dogs for this disease in our study.

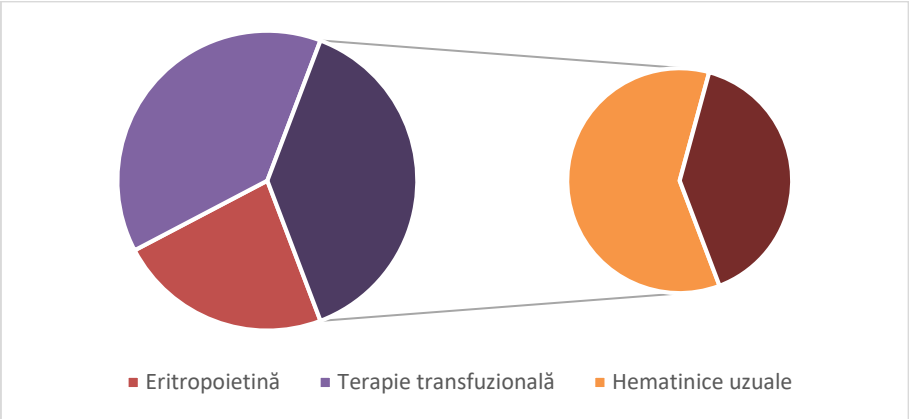

Fig. 6. Graphic representation of the use of the main therapeutic protocols in anemia used in our study

Regarding the therapeutic protocols (Fig. 6) used, 5 patients were treated with erythropoietin (33.3%), 7 of them received transfusion therapy (55.5%), and 8 of them received usual hematinics (66.6%). Regarding transfusion therapy, 4 of the individuals (40%) received red cell concentrate, while the remaining 3 cases (60%) received whole blood.

Conclusions

The investigations were carried out on a number of 30 patients with anemia, of which 12 were represented by animals belonging to the feline species (40%) and 18 cases by animals of the canine species (60%).

The cases included in the present study recognized different causes, especially determined by autoimmune hemolytic anemia, chronic renal failure and rodenticide poisoning, and other conditions.

Incidentally, the numerical and percentage distribution according to sex, recorded a preponderance of conditions dominated and/or accompanied by slightly higher anemia in males (53%), compared to females (47%).

Correlating the evaluation of the presence and degree of anemia and early curative intervention adapted to the cause, allowed the establishment of a diagnostic algorithm and an appropriate therapeutic protocol.

References

1. **Allen D., Kruth S., Garvey M.,** Small Animal Medicine, Ed. J.B. Lippincott Co., USA, 1991.
2. **Bonagura, J.D.,** Kirk's Current Veterinary Therapy XV, 1st Edition, Elsevier, 2014.
3. **Bradley, K.,** Cunningham's Textbook of Veterinary Physiology, Saunders Elsevier, Blacksburg, VA, SUA, 1992.
4. **Bradley, K.,** Cunningham's Textbook of Veterinary Physiology, Saunders Elsevier, Blacksburg, VA, SUA, 2019.
5. **Chakrabarti, A.,** Textbook of Clinical Veterinary Medicine, Kalyani Publishers, 2014.
6. **Codreanu, M.D.,** Terapeutică veterinară", manual anul VI, Ed. Printech, 2014.
7. **Codreanu, M.D.,** Tratat de ultrasonografie veterinară, Ed. Medicală, 2021.
8. **Codreanu, M.D., Fernoagă, C.,** Veterinary internal medicine, Ed. Terra aurum, 2020.
9. **Codreanu, M.D.,** Patologie şi clinică medicală veterinară, Ed. Printech, ediţie adăugită/revizuită, 2020.
10. **Codreanu, M.D.,** Veterinary internal medicine, Ed. Terra aurum, 2020.
11. **Codreanu, M.D.,** Veterinary therapeutics, Ed. Terra aurum, 2020.
12. **Crista, N., Boişteanu, I., Bârză, E., Barburat, A.,** Fiziologia animalelor domestice, Editura Didactică şi Pedagogică, Bucureşti, 1978.
13. **Crivineanu, V., Crivineanu, M., Stătescu, C. Codreanu, M.,** "Farmacoterapie, farmacotoxicologie şi farmacovigilenţă", Editura Fundaţiei "România de mâine", Bucureşti, 1999.
14. **Ettinger, S.J., Feldman, E.C.,** Textbook of Veterinary internal medicine expert consult (eighth edition), Saunders Elsevier, 2017.
15. **Ettinger, S.J., Feldman, E.C.,** Textbook of Veterinary Internal Medicine, vol. II, Ed. Saunders, 2010.
16. **King, L.G., Boag, A.,** BSAVA Manual of Canine and Feline Emergency and Critical Care, (third), British Small Animal Veterinary Association, Quedgeley, Gloucs, England, 2018.
17. **Mizuno, T., Inoue, M., Kubo, T., Iwaki, Y., Kawamoto, K., Itamoto, K., Kambayashi, S., Igase, M., Baba, K., Okuda, M.,** Improvement of anemia in five dogs with nonregenerative anemia treated with allogeneic adipose-derived stem cells, Veterinary Animal Sciences, 2022, 17, 100264.
18. **Naigamwalla, D.Z., Webb, J.A., Giger, U.,** Iron deficiency anemia, Canadian Veterinary Journal, 2012, 53, 3, 250-6.
19. **Solcan, Gh., Boghian, V., Rollin, F.,** Patologie și clinică medicală veterinară, Ed. Ion Ionescu de la Brad, Iași, 2005.
20. **White, C., Reine, N.,** Feline nonregenerative anemia: pathophysiology and etiologies, The Compendium on continuing education for the practicing veterinarian, 2009, 31, 7, 1-7.

THERAPEUTIC EFFICACY ASSESSMENT OF OXYCLOZANIDE AGAINST PARAMPHISTOMUM IN CATTLE

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Summary

Cattle are one of the most important species of domestic animals raised in Romania. Parasitic elements identified in cattle are very diverse and common. The aim of this study was to determine the efficacy of oxyclozanide against paramphistomosis in cattle under conditions of natural infestation. The cattle taken into study were randomly divided into two groups of 10 individuals. One group was the untreated control group and the other group was treated with oxyclozanide administered orally at a dose of 20 mg/kg body weight. Feces were collected from each animal from the rectum on days 0, 7, 14, 21 and 28 and examined using the McMaster method. The efficacy of oxyclozanide was 91% on day 7 and 100% on days 14, 21 and 28. In conclusion oxyclozanide has proved effective in treating *Paramphistomum* spp. in cattle.

Keywords: cattle, EPG, efficacy, oxyclozanide.

Parasitism is one of the major problems decreasing the productivity of livestock worldwide (21). The importance of helminth infection is increasing in developing countries (19). Around the world, vast amounts of money are spent every year to control helminths in animals (8). The epidemiology of the disease is closely associated with environmental factors, temperature, humidity, precipitation, and the ecology and infection of the intermediate host snail, *Lymnaea* spp. (10). Parasitic diseases are responsible for significant economic losses due to the negative effect on animal health and productivity, feed conversion, growth rate, milk production and quality, reduced reproductive capacity and reduced weaned calf weight (11).

Paramphistomum infection causes lower feed conversion, weight loss and/or reduced milk production, leading to economic losses (17).

Paramphistomum cervi is considered to be one of the most important paramphistoma species, with a cosmopolitan distribution (3, 7). Paramphistomosis can be controlled by regular treatment. Several drugs have been evaluated and recommended for the treatment of paramphistomosis (14).

The present study focused mainly on evaluating the effect of oxyclozanide on paramphistomes parasitizing in cattle.

Materials and methods

The study was carried out in Traian Vuia village between July and October 2022. Twenty cattle naturally infected with *Paramphistomum* spp. were detected

using the sedimentation method described by Soulsby (20). Fecal samples were collected directly from the rectum in sterile containers and kept refrigerated until they were transported in cold boxes to the Parasitology and Parasitic Diseases Department of the Faculty of Veterinary Medicine in Timisoara.

The cattle in the study were randomly divided into two groups of 10 cattle. One group was the untreated control and the other group was treated with oxyclozanide administered orally at a dose of 20 mg/kg body weight. Individual faeces were collected from each bovine rectum on days 0, 7, 14, 21 and 28 and examined by the McMaster method.

The anthelmintic efficacy rate was determined by applying the FECRT according to the relationship of President (%) (16):

$$1 - \left(\frac{T2}{T1} x \frac{C1}{C2}\right) x\ 100, \text{ where:}$$

T1 – EPG treated group day 0;
T2 – EPG treated group day 10;
T3 – EPG control group day 0;
T4 – EPG control group day 10.

Results and discussions

Previous data on the safety of oxyclozanide administration in cattle and sheep showed that relatively low single doses (15 mg/kg) could have adverse effects on the central nervous system and intestinal function (behavioural depression, diarrhoea and anorexia) (5). In addition, severe signs of toxicity and mortality have been reported at doses ≥50 mg/kg (5). However, in the present study, no adverse effects were observed at a dose of 20 mg/kg administered twice in cattle.

The mean EPG from 0 to 28 days of treatment of oxyclozanide-treated and untreated cattle is presented in Table 1. Similarly, the trend of reduction in faecal egg counts as a function of treatment and control days is presented in Fig. 1. The average EPG decreased significantly each week after treatment.

Tabel 1

Fecal egg count at different time intervals

GROUPS	EPG DAY 0	EPG DAY 7	EPG DAY 14	EPG DAY 21	EPG DAY 28
UNTREATED CONTROL	**450**	**500**	**550**	**500**	**550**
OXYCLOZANIDE	**500**	**50**	**0**	**0**	**0**

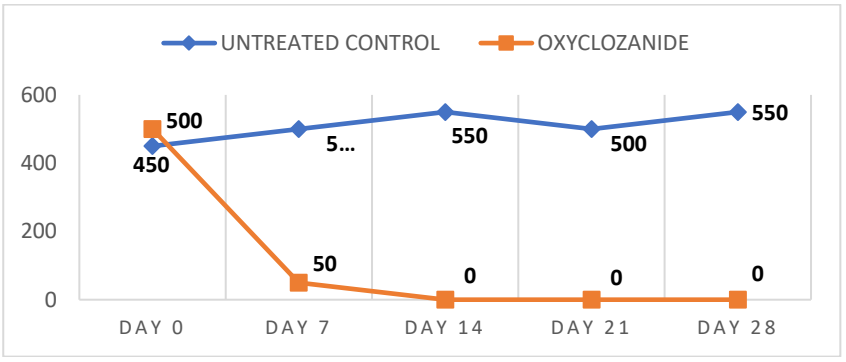

Fig. 1. Effect of oxyclozanide on Fecal Egg Count Reduction

Following treatment in Traian Vuia cattle, oxyclozanide was found to be effective in reducing EPG at a dose of 20mg/kg body weight. The efficacy of oxyclozanide was 91% on day 7, and on days 14, 21 and 28, efficacy was 100% (Fig. 2).

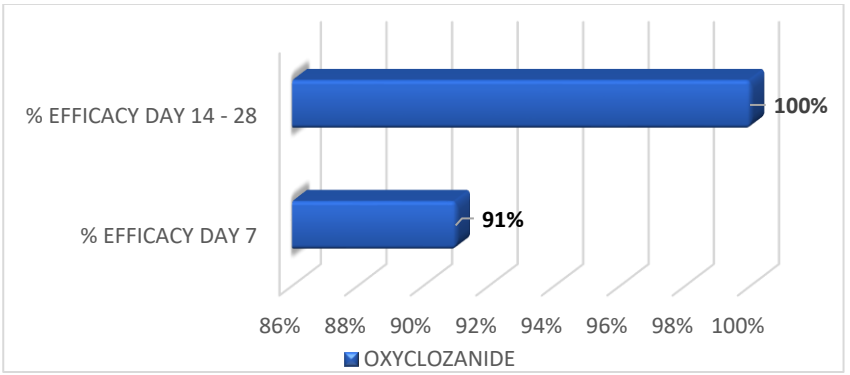

Fig. 2. Graphical representation of effectiveness on day 7 and days 14 - 28

Oxyclozanide has been reported to be the best anthelmintic against paramphistomosis in cattle (4, 12).

This study shows that significant reduction in EPG in the treatment group receiving 20 mg/kg body weight from day 7 to day 28, which is supported by Osman et. Al. (13) in cattle from Sudan, Aries (2) in cattle from Spain and Acharya et al. (1) in cattle from Nepal. However, in the case of triclabendazole, Ghimire (6) has shown a lower percentage of FECRT in cattle.

Other data recorded by Rolfe and Boray (18) reported that oxyclozanide administered at a dose of 18.7 mg/kg reduced the number of parasites (*Calicophoron*

calicophorum) in the small intestine, abomasum, rumen and grid by 61 to 96.1%, 50.0 to 92.6%, and 56.5 to 98.1%, respectively. By administering 2 doses at 3 days apart, oxyclozanide was 99.9%, 100 and 100% effective and produced improvements in clinically affected calves.

Conclusions

In conclusion, oxyclozanide administered orally to cattle twice has been demonstrated to be safe with no adverse reactions detected in treated animals. This two-dose schedule is preferred not only because of its efficacy, but also because of the variable results that single treatments have shown in some reports (9, 15, 18).

References

1. **Acharya, M.P., Pandey, G., Bastakoti, R., Chapagain, P.B., Sah, A.K., Pant, S.R., Acharya, B.R., Ghimire, S.H., Ghimire, S., Pandeya, Y.R., Hamal, P., Pathak, P.H.L.,** Efficacy of oxyclozanide against *Paramphistomum* (rumen fluke) in cattle of Madi Valley Chitwan, Proceedings of the 12th National Outreach Research Workshop, 2017, 18, 19.
2. **Arias, M.S., Sanchís, J., Francisco, I., Francisco, R., Piñeiro, P., Cazapal-Monteiro, C., Paz-Silva, A.,** The efficacy of four anthelmintics against *Calicophoron daubneyi* in naturally infected dairy cattle, Veterinary Parasitology, 2013, 197, 1, 126-129.
3. **De Waal, T.,** *Paramphistomum* - a brief review, Irish Veterinary Journal, 2010, 63, 5, 313-315.
4. **Díaz, P., Lomba, C., Pedreira, J., Arias, M., Sánchez-Andrade, R., Suárez, J.L., Díez-Baños, P., Morrondo, P., Paz-Silva, A.,** Analysis of the IgG antibody response against *Paramphistomidae* trematoda in naturally infected cattle, Application to serological surveys Veterinary Parasitoogy, 2006, 140, 281-288.
5. **EMEA,** The European Agency for the Evaluation of Medicinal Products Veterinary Medicines and Inspections, EMEA/MRL/889/03-FINAL, Committee for Veterinary Medical Products, Oxyclozanide, Summary report (3), 2004.
6. **Ghimire, N.P., Karki, N.P.S.,** Prevalence of Fasciolosis and efficacy of various anthelmentics in buffaloes of rural Kathmandu, Proceedings of the 5th National veterinary Conference, Nepal, 1996, 24, 108111.
7. **Hassan, S.S., Kaur, K., Juyal, P.D.,** Epidemiology of paramphistomosis in domestic ruminants in different districts of Punjab and other adjoining areas, Journal of Veterinary Parasitology, 2005, 19, 43-46.
8. **Jabber, M., Green, D.A.G.,** The status and potential of livestock within the context of agricultural development policy in Bangladesh, The University of wales, Aberystwyth, United Kingdom, 1983, 113.
9. **Jeyathilakan, N., Radha, G., Gomathinayagam, S., Senthilvel, K., Lalitha, J.,** Reduced efficacy of oxyclozanide against amphistomes in naturally infected sheep, Indian Journal of Small Ruminants, 2005, 11, 96-97.

10. **Lloyd, J., Boray, J., Love, S.,** Stomach fluke (*Paramphistomes*) in ruminants, Primefact, 2007.
11. **Mehlhorn, H.,** Encyclopedia of Parasitology Volume 2: N-z; Energy-Metabolism Disturbing Drugs, 3rd Ed. Springer-Verlag Berlin, 2008.
12. **Murphy, T.M., Power, E.P., Sánchez-Miguel, C., Casey, M.J., Toolan, D.P., Fagan, J.G.,** Paramphistomosis in Irish cattle, Veterinary Record, 2008, 162, 831.
13. **Osman, O.,** The efficacy of tricalbendazole and oxyclozanide against natural *Fasciola gigantica* infection in cattle, Sudan Journal of Veterinary Research, 2013, 28, 37-42.
14. **Panyarachun, B., Sobhon, P., Yotsawan Tinikul, Y., Chotwiwatthanakun, C., Anupunpisit, V., Anuracpreeda, P.,** *Paramphistomum cervi:* surface topography of the tegument of adult fluke, Experimental Parasitology, 2010, 125, 95-99.
15. **Paraud, C., Gaudin, C., Pors, I., Chartier, C.,** Efficacy of oxyclozanide against the rumen fluke *Calicophoron daubneyi* in experimentally infected goats, Veterinary Journal, 2009, 265-267.
16. **Presidente, P.J.A.,** Methods for detection of resistance to anthelmintics. CSIRO Division of Animal Health, Glebe, NSW, Australia, 1985, 13-28.
17. **Rangel-Ruiz, L., Albores-Brahms, S., Gamboa-Aguilar, J.,** Helmintholmal Parasites: Their Life Cycles and Ecology. Seasonal trends of *Paramphistomum cervi* in Tabasco, Mexico, Veterinary Parasitology, 2003, 116, 3, 217-222.
18. **Rolfe, P.F., Boray J.C.,** Chemotherapy of paramphistomosis in cattle, Australian Veterinary Journal, 1987, 64, 328-332.
19. **Rondelaud, D., Vignoles, P., Abrous, M., Dreyfuss, G.,** The definitive and intermediate hosts of *Fasciola hepatica* in the natural watercress beds in central France, Parasitology Research, 2001, 87, 6, 475-478.
20. **Soulsby, E.J.L.,** Helminths, arthropods and protozoa of domestic animals, 7th edition, W.B. Saunders, Baillere Tindall, London, 1982.
21. **Vercruysse, J., Claerebout, E.,** Treatment vs non-treatment of helminth infections in cattle: defining the threshold, Veterinary Parasitology, 2001, 98, 1, 195-214.

MITOCHONDRIA - AN ESSENTIAL COMPONENT OF THE OOCYTE

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Summary

Oocyte maturation represents the successfully end of the complex process of growth and development occurring in the ovarian follicle. Fully grown oocytes become able of undergoing the three main aspects of maturation: nuclear, epigenomic and cytoplasmic. These three processes must be achieved simultaneously. In addition, oocyte maturation directly depends on the ability of mitochondria to ensure bioenergetic reserves. These are highly dynamic organelles that constantly move, fuse and divide in response to the various energy demands of the cell, the dynamics being mediated by large dynamin-GTPases (DRPI, OPAI, MFN1 and MFN2) contained in mitochondrial membranes. Optimal mitochondrial activity relates to mitochondrial structural changes, mutation load, available mtDNA copy number, as well as mitochondrial motility. Mitochondria produce reactive oxygen species (ROS) which are the toxic residues resulting from the OXPHOS process. These cell organelles integrate temporary permeability pores (mtPTPs) that initiate cell death through their opening when the energy-generating function of the mitochondria declines. As a result, mitochondrial dysfunctions that will inhibit OXPHOS and allow the production of higher amounts of ROS will lead to apoptosis. Mitochondrial functions can be affected by increased ROS levels. Mitochondrial oxidative phosphorylation is the primary source of ROS. The importance of ensuring a minimum threshold number of mitochondria that ensures developmental capacity has determined the use of medicinal agents to increase mitochondrial mass or to improve mitochondrial function. Thus, this study includes information related to antioxidant substances, such as *alpha-ketoglutarate*, *resveratrol*, *CoQ10* and how they act on amplifying the functioning of mitochondria and suppressing oxidative stress at the level of oocytes.

Keywords: mitochondria, oocyte, apoptosis.

Mitochondria are essential for oocyte maturation, fertilization and embryonic development. Optimal mitochondrial activity relates to mitochondrial structural changes, mutation load, available mtDNA copy number, as well as mitochondrial motility, a property that allows them to move to the right places to ensure the energy level required by local activities. Affected mitochondria will significantly decrease ATP synthesis in oocytes which will negatively affect division axis formation, chromosome separation and fertilization, resulting in decreased oocyte quality and aneuploidy. Since mitochondria are passed on to embryos through the mother, there is the possibility of passing mutations or deletions to the next generation. Offspring lifespan is hypothesized to be affected by maternal age, an effect that appears to be influenced by maternal mtDNA inheritance as well as epigenetic factors (11).

Mitochondria are involved in so called intrinsic apoptotic pathways in which they release soluble proteins including cytochrome C from the intermembrane space to initiate caspase activation in the cytosol. The release of some proteins is the result of damage to the integrity of the outer mitochondrial membrane, a process called outer mitochondrial membrane permeabilization (14).

Mitochondria is a cellular organelle that is found in all eukaryotic cells. It produces ATP and it is involved in calcium homeostasis and apoptosis. It consists of two membranes. The outer one separates the mitochondrial matrix from the cellular cytoplasm and the inner one forms the cristae. The inner one is the seat of the ETC (electron transport chain) and the place where the final stage of cellular respiration takes place, namely oxidative phosphorylation (14).

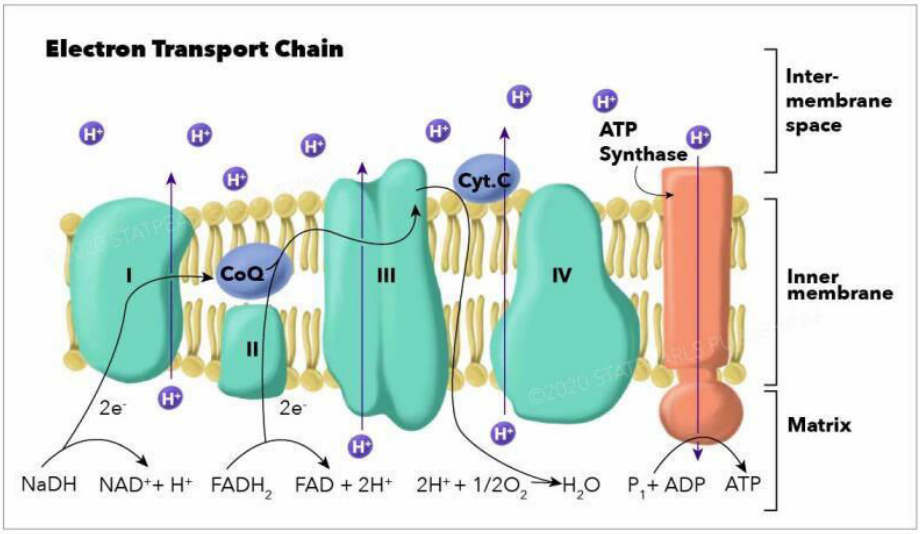

Fig. 1. ETC chain and ATP generation (10)

Oocyte maturation

Oocyte maturation represents the successfully end of the complex process of growth and development occurring in the ovarian follicle. Fully grown oocytes become able of undergoing the three main aspects of maturation: nuclear, epigenomic and cytoplasmic. These three processes must be achieved simultaneously (7).

Nuclear maturation involves breaking out of the meiotic block in dictyotene (germinal vesicle stage) and advancing meiosis from prophase I to metaphase II (M II). The fulfillment of the epigenomic side depends on the initiation, course and finalization of covalent epigenetic modifications in terms of the extension of the methylation process among the cytosine residues of the genomic DNA. At the same

time, visible changes occur in the processes of deacetylation, methylation/demethylation of lysine and arginine contained in histone nucleosomes (4).

Cytoplasmic maturation involves the accumulation of mRNA, proteins and nutrients, as well as the redistribution of organelles indispensable for reaching meiotic competence, completing the process of meiotic maturation, and finally reaching the M II oocyte stage (4).

The completion of oocyte maturation is conditioned by the existence of cumulus cells characterized by a very good molecular quality. The intrafollicular compartments made up of the oocyte and the cumulus cells work together to ensure the bidirectional exchange of small molecules (ATP, cAMP and calcium ions) that takes place through the gap junctions. Optimal functioning of cumulus cells allows maintenance of meiotic arrest followed by participation in the resumption of meiosis and support of oocyte maturation (7).

In addition, oocyte maturation directly depends on the ability of mitochondria to ensure bioenergetic supplies. On one hand, mitochondria are considered the power plant of each cell as well as bioaccumulators that store the bioenergy resulting from ATP synthesis. On the other hand, these organelles are also responsible for the regulation of redox reactions, intracellular calcium homeostasis, as well as the control of apoptotic processes – especially through the transcription factor FOXO3, which regulates the mitochondrial apoptotic pathway (8).

The action of various factors on the competence of the oocyte

Different stress factors (mother's nutrition, age of the female, toxins, inflammatory conditions, thermal stress) can negatively affect the oocyte as it acquires its ability to develop during the long process of folliculogenesis. Therefore, exposure of the body to any of the stress factors can lead to a decrease in the competence of maturation and then fertilization of the oocyte contained in the follicle. Mitochondria are a prime target for stressors (15).

Oocytes collected from cows during summer have a lower fertilizing capacity and possibility of reaching the blastocyst stage compared to those obtained in winter. Furthermore, a period of two or three sexual cycles is required for oocytes with normal developmental capacity to appear suggesting a carryover effect from the oocyte to the developing embryo stage. (16) Thermal stress affects the proper development of microtubulin and microfilaments of the cytoskeleton, disruption of the division axis as well as a reduced number of oocytes progressing to M II (14, 15).

The induced changes in the cytoskeleton are correlated with the desynchronization of cytoplasmic maturation events such as translocation of cortical granules as well as mitochondrial distribution (7). Heat stress affects RNA storage at the germinal vesicle stage and dysregulates transcription in mature oocytes. (5)

The reduced developmental capacity of oocytes during summer is related with changes in mitochondrial characteristics over the seasons. Mitochondrial

transport and repositioning in cells occurs along microtubule trajectories as well as by rearrangement of actin filaments (20).

Oocytes can be classified based on the distribution of mitochondria into four categories: 1. Mitochondria are arranged in homogeneous groups distributed in the cytoplasm of the oocyte; 2. Groups of restricted mitochondria located at the periphery of the oocyte; 3. The existence of a small number of peripherally arranged mitochondria; 4. Non-viable oocytes (6).

There is an obvious correlation between the distribution of mitochondria and the polarity of the mitochondrial membrane, category 1 having an increased polarity and category 3, a decreased one (3).

Since maternal mitochondria stored in the oocyte are the primary source of energy for later embryonic development, these changes may explain the delay in divisions to the 2 or 4 cell stages as well as the reduced proportion of blastocysts resulting from warm-season harvested oocytes (7).

The effects of antioxidant substances on the functioning of mitochondria and the suppression of oxidative stress in oocytes

Aerobic metabolism is associated with the production of prooxidant molecules called free radicals or reactive oxygen species. These include the hydroxyl radicals, hydrogen peroxide, superoxide anion and nitric oxide. There is a complex interaction between prooxidants and antioxidants that results in the maintenance of intracellular homeostasis. When there is an imbalance between prooxidants and antioxidants, a state of oxidative stress is initiated (11).

Free radicals can influence oocytes, spermatozoa and embryos in their microenvironments, for example follicular fluid, hydrosalpingeal fluid and peritoneal fluid. These microenvironments are directly related to oocyte quality, oocyte-sperm interaction, implantation and early embryo development. There is a complex interplay of cytokines, hormones and other stress factors that affect cellular generation of free radicals; these molecules further act by modulating many transcription factors and gene expression (11).

Coenzyme Q10 (CoQ10), which consumes free radicals and is an essential element of the mitochondrial respiratory chain can be added to the culture medium to counteract the effects of ROS. Incorporation of CoQ10 into oocytes induces changes in gene transcription leading to an increase in the percentage of embryos that reach the blastocyst stage (7, 17).

Detrimental effects of oxygen-derived free radicals during in vitro culture (IVC) have been demonstrated in several species. ROS have been reported to induce mitochondrial dysfunction, damage to DNA, RNA and proteins, as well as inhibition of sperm-oocyte fusion. To protect oocytes and embryos from oxidative stress during IVC, various antioxidants can be added to the culture media (18).

For example, IVC media supplemented with SOD exerted a protective effect against oxidative stress on the development of fertilized mouse embryos in vitro and in vivo. Supplementation of protein-free culture medium with superoxide dismutase

proved to increase the proportion of rabbit zygotes developing to the expanded blastocyst stage (18).

However, other authors reported that the proportion of bovine oocytes developing to morula and blastocyst stages was not improved when in vitro maturation (IVM) and culture medium were supplemented with SOD. In addition, it has been shown that the expression of embryonic SOD-mRNA is affected by the culture conditions used (12, 13).

Supplementation of mitochondria in mtDNA-deficient sow oocytes with mitochondria isolated from M II stage oocytes promoted mtDNA replication prior to embryonic genome activation, also enhancing blastocyst development (1).

It is also possible to add metabolizable antioxidants to the culture media, instead of enzymatic antioxidants. Glutathione (GSH) is a natural antioxidant present in both gametes, but its level varies. An increase in intracellular GSH concentration is demonstrated as oocytes progress from the germinal vesicle to the metaphase II stage, but GSH is lower in fertilized oocytes at the pronuclear stage compared to mature oocytes (4, 19).

Moreover, GSH plays an important role in protecting the cell against oxidative damage and in the formation of the male pronucleus in the hamster, mouse, and pig. Thus, GSH levels found in oocytes at the end of maturation are considered a good biochemical marker for oocyte viability (17).

By adding *alpha-ketoglutarate* (metabolite in the tricarboxylic acid cycle), a powerful antioxidant to the oocyte culture medium, Chen et al. (2) obtained the attenuation of the expression of Bcl2 and p53 genes as well as the enhancement of the expression in the porcine embryo of genes associated with pluripotency (OCT4, NANOG and SOX2) (2).

Conclusions

Mitochondria, a cellular organelle that produces ATP is involved in calcium homeostasis and apoptosis.

Mitochondria are essential for oocyte maturation, fertilization and embryonic development.

Based on the distribution of mitochondria and on their ability to ensure bioenergetic supplies, oocytes can be classified into four categories.

Regarding the steps where they act in the respiratory chain, various antioxidants can be added to the culture medium to counteract the effect of reactive oxygen species and to maintain the propper redox level.

References

1. **Cagnone, G.L.M., Tsai, T.S., Makanji, Y., Matthews, P., Gould, J., Bonkowski, M.S., Elgass, K.D., Wong, A.S., Wu, L.E., McKenzie, M., Sinclair, D.A., St John, J.C.,** Restoration of normal embryogenesis by mitochondrial supplementation in pig oocytes exhibiting mitochondrial DNA deficiency, Scientific Reports, 2016, 6, 23229.
2. **Chen, Q., Gao, L., Li, J., Yuan, Y., Wang, R., Tian, Y., Lei, A.,** α- Ketoglutarate Improves Meiotic Maturation of Porcine Oocytes and Promotes the Development of PA Embryos, Potentially by Reducing Oxidative Stress through the Nrf2 Pathway, Oxidative Medicine and Cellular Longevity, 2022, 1-17.
3. **Chiaratti, M.R., Garcia, B.M., Carvalho, K.F., Macabelli, C.H., Ribeiro, F.K.D.S., Zangirolamo, A.F., Sarapião, F.D., Seneda, M.M., Meirelles, F.V., Guimarães, F.E.G., Machado, T.S.,** Oocyte mitochondria: role on fertility and disease transmission, Animal Reproduction, 2018, 3, 231-238.
4. **Dai, J., Wu, Caroline, C., Muneri, W., Niu, Y., Zhan, S., Rui, R., Zhang, D.,** Changes in mitochondrial function in porcine vitrified MII-stage oocytes and their impacts on apoptosis and developmental ability, Criobiolgy, 2015, 71, 291-298.
5. **De Matos D.G., Furnus, C.C.,** The importance of having high glutathione (GSH) level after bovine in vitro maturation on embryo development effect of beta-mercaptoethanol, cysteine and cystine, Theriogenelogy, 2000, 53, 3, 761-71.
6. **Ferreira, R.M., Chiaratti, M.R., Macabelli, C.H., Rodrigues, C.A., Ferraza, M.L., Watanabe, Y.F., Smith, L.C., Meirelles, F.V., Baruselli, P.S.,** The infertility of repeat breeder cows during summer is associated with decreased mitochondrial DNA and increased expression of mitochondrial and apoptotic genes in oocytes, Biology of Reproduction, 2016, 94, 66.
7. **Gao, W., Jin., Y., Hao., J., Huang., S., Wang, D., Quan F., Ren, W., Zhang, J., Zhang, M., Yu, X.,** Procyanidin B1 promotes in vitro maturation of pig oocytes by reducing oxidative stress, Molecular Reproduction and Development, 2021, 88, 55-66.
8. **Gendelman, M.**, **Roth, Z.,** Incorporation of Coenzime Q10 into Bovine Oocytes Improves Mitochondrial Features and Alleviates the Effects of Summer Thermal Stress on Developmental Competence, Biology of Reproduction, 2012, 87, 5, 118, 1-12.
9. **Gorczyca, G., Wartalski, K., Romek, M., Samiec, M., Duda, M.,** The Molecular Quality and Mitochondrial Activity of Porcine Cumulus-Oocyte Complexes Are Affected by Their Exposure to Three Endocrine-Active Compounds under 2D In Vitro Maturation Conditions, International Journal of Molecular Sciences, 2022, 23, 9, 4572.
10. **Hammond, E.R., Green, M.P., Shelling, A.N., Berg, M.C., Peek, J.C., Cree, L.M.,** Oocyte mitochondrial deletions and heteroplasmy in a bovine model of

ageing and ovarian stimulation, Molecular Human Reproduction, 22, 2016, 261-271.

11. **Kageyama, M., Ito, J., Shirasuna, K., Kuwayama, T., Iwata, H.,** Mitochondrial reactive oxygen species regulate mitochondrial biogenesis in porcine embryos, Journal of Reproduction and Development, 2021, 67, 141-147.
12. **Kala, M., Shaikh, M.V., Nivsarkar, M.,** Muhammad Vaseem Shaikh, Manish Nivsarkar, Equilibrium between anti-oxidants and reactive oxygen species: a requisite for oocyte development and maturation, Reproductive Medicine and Biology, 2016, 16, 28-35.
13. **Li, H.**, **Chian, R.C.,** Mitochondrial Dysfunction and Age-related Oocyte Quality, Reproductive and Developmental Medicine, 2017, 1, 45-54.
14. **May-Panloupe P.**, **Boucret L., Chao de la Barca, J.M., Desquiret-Dumas, V., Ferré-L'Hotellier, V., Morinière, C., Descamps, P., Procaccio, V., Reynier, P.,** Ovarian ageing: the role of mitochondria in oocytes and follicles, Human Reproduction Update, 2016, 22, 6, 725-743.
15. **Perreault S.D., Barbee, R.R., Slott, V.L.,** Importance of glutathione in the acquisition and maintenance of sperm nuclear decondensing activity in maturing hamster oocytes, Developmental Biology, 1988, 125, 181-186.
16. **Roth, Z.,** Cellular and molecular mechanisms of heat stress related to bovine ovarian function, Journal of Animal Science, 2015, 93, 5, 2034-2044.
17. **Roth, Z.,** Reduction in oocyte developmental competence by stress is associated with alterations in mitochondrial function, Journal of Dairy Science, 2018, 101, 3642-3654.
18. **Sato, D., Itami, N., Tasaki, H., Takeo, S., Kuwayama, T., Iwata, H.,** Relationship between mitochondrial DNA Copy Number and SIRT1 Expression in Porcine Oocytes, PLoS ONE, 2014, 48, 61-73.
19. **Yang, C-X., Liu, S., Miao, J.K., Mou, Q., Liu, X.M., Wang, P.C., Huo, L.J., Du, Z.Q.,** CoQ10 improves meiotic maturation of pig oocytes through enhancing mitochondrial function and suppressing oxidative stress, Theriogenology, 2021,159, 77-86.
20. **Zampolla, T., Spikings, E., Rawson, D., Zhang, T.,** Cytoskeleton proteins F-actin and tubulin distribution and interaction with mitochondria in the granulosa cell surrounding stage III zebrafish (Danio rerio) oocytes, Thriogenology, 2011, 76, 1110-1119.

ETIOTROPIC MANAGEMENT OF NEONATES DIARRHEA IN LAMBS

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Summary

In the intensive breeding of farm animals, the neonatal period constitutes a critical window of maximum vulnerability due to the lack of an own functional immunology system and dependence on passive immunity - acquired through absorption colostrum immunoglobulins, in the first 24 hours of life. The lot included 40 lambs, of which 18 were males and 22 females, from 25 ewes. In this group, 12 animals developed the symptoms of neonatal diarrhea syndrome, thus, it was decided to divide it into two subgroups, further noted 7A and 7B, each of which includes 6 individuals, aged between 3-6 days of life. The diagnosis was done with the Rainbow Bio K 316 rapid test, resulting in a pronounced infection with Cryptosporidium parvum. The therapeutic management of the subgroups involved the administration in the form of protocols of Paromomycin at a dose of 100mg/kg GC, per os, once a day, for 11 days and Halofuginone at a dose of 100mg/kg per os, once a day, for 7 days. The approach of a correlated etiological treatment, after the identification of the primary responsible pathogen agent, and the implementation of a modular therapy, individually adapted, allowed the reduction of mortality of young sheep which present peri- and neonatal diarrhea syndrome.

Keywords: lambs, diarrhea, neonatal, therapy.

In the induction of this syndrome, a plurifactorial etiological ensemble is incriminated, the etiological agents acting against the background of the existence of favorable conditions (different from actual to actual and with various implications) (2, 8).

Noninfectious etiology includes predisposing factors that, in correlation with the presence of etiologically infectious agents, determine the symptomatology of neonatal diarrhea syndrome (6, 11). Predisposing factors include maternal, nutritional, immunological, genetic and zoological (7).

Along with noninfectious etiology, production management unrelated to the level of prophylactic standards, various pathogens compete for the materialization of the complex symptomatology included under the name peri and neonatal diarrhea syndrome of the youth of farm animals (8, 9).

The infectious component was systematized into three distinct groups in accordance with the characteristics of the etiological agent: a viral, a bacterial and a parasitic category (1, 9).

The group of infectious agents of viral origin includes, *Rotavirus spp*. and *Bovine Coronavirus*, and recent studies indicate the involvement of some viruses

from the Families: *Astroviridae, Picornaviridae and Calciviridae*. For viral etiology, treatment options are limited to general animal support methods, without an etiotropic approach in dynamics (4, 14).

Infectious microbial agents have a notable impact in the pathology of neonatal diarrhea syndrome, this category including: *Escherichia coli*, *Clostridium perfringens* and *Salmonella spp*. (10, 12, 13).

The parasitic etiology is dominated by *Cryptosporidium parvum* along with *Eimeria spp*. and *Giardia duodenalis* (3, 15, 16, 18).

Knowing the etiological agents requires an individual modulation of treatment, an etiotropically directed approach that has a high effectiveness. (5, 17).

Regarding the parasitic pathology caused by *Cryptosporidium parvum*, the therapeutic approach mentioned in the bibliographic data specifies that the only molecules with proven effectiveness against cryptosporidiosis are halofuginone and paromomycin (2, 19, 20).

Materials and methods

The lot included 40 lambs, of which 18 were males and 22 females, from 25 ewes. In this group, 12 animals developed the symptoms of neonatal diarrhea syndrome, thus, it was decided to divide it into two subgroups, further noted 7A and 7B, each of which includes 6 individuals, aged between 3-6 days of life. The diagnosis was done with the Rainbow Bio K 316 rapid test, resulting in a pronounced infection with *Cryptosporidium parvum*. (Fig. 1, Fig. 2, Fig. 3, Fig. 4)

Fig. 1. FecalSwab sampling

Fig. 2. FecalSwab sampling

The clinical signs included lethargy, depressed mental status, inert behavior to the action of environmental factors, disappearance of the sucking reflex, severe

dehydration watery diarrhea, with frothy, light yellow color, hypothermia (36-36.3°C) and dyspnea (Fig. 2, Fig. 7).

The diagnosis was done with the Rainbow Bio K 316 rapid test, resulting in a pronounced infection with *Cryptosporidium parvum* (Fig. 5, 6).

Fig. 3. Feces sample

Fig. 4. Result reading of Rainbow rapid test

Valori in afara limitelor admise pentru varsta si sexul respectiv

Denumire	Rezultat	UM	Interval de referinta
Parazitologie			
Examen microscopic Cryptosporidium spp.			
Prezente oochisturi de Cryptosporidium spp. pe frotiurile examinate.			

Fig. 5. Positive result of *Cryptosporidium parvum* in Rainbow Bio k 316 rapid test

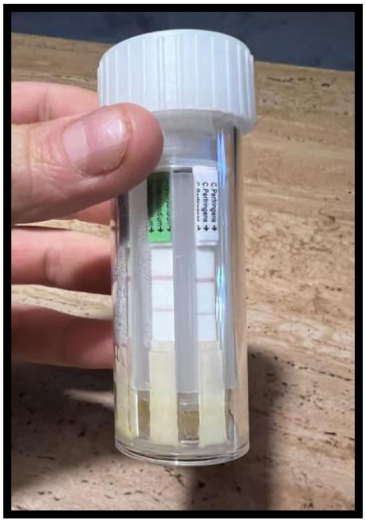

Fig. 6. Batch 6 rapid test result

Fig. 7. Profuse diarrhea in a lamb

Results and discussions

The therapeutic management of the subgroups involved the administration of the following medicinal substances in the form of protocols:

- Lot 7A are administered paromomycin (commercial product Gabrovet/CEVA) at a dose of 100mg/kg GC, per os, once a day for 11 days.
- Lot 7B is given halofuginone (commercial preparation Halocur - Intervet) in a dose of 100mg/kg orally, once a day, for 7 days.

The common drug therapeutic approach included:

- Buscopan 0.15-0.2 ml intramuscularly, one administration per day, the first 3 days.

- Venous rehydration with 1.4% sodium bicarbonate solution, repeated administrations of 80ml, alternating with 5% dextrose solution at doses of 4mg/kg/min (Fig. 8).

- Flunixin in a dose of 1mg/kg intramuscularly, single administration in cases of discomfort.

Combating hypothermia was achieved by placing the lambs in the incubator.

The dietary therapy approach involved the withdrawal of the lambs from their mothers, the milk being replaced by astringent teas and qualitative milk substitute, customized to the needs of the sheep species, and the administration being assisted and controlled (Fig. 9) for 7 days, during which it was gradually reintroduced milk in food.

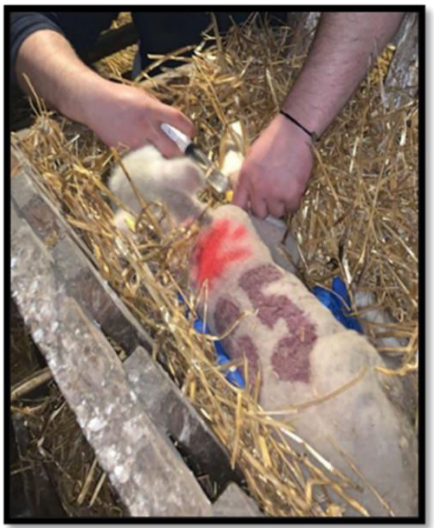

Fig. 8. Rehydration through the cephalic vein approach

Fig. 9. Assisted feeding

The evolution is favorable and similar in both subgroups. One mortality was noted in batch 7B.

The corroboration of data from the field, observed during the study, come as proven factors that can be the basis for optimizing the correlated etiological treatment in peri and neonatal diarrhea in young sheep.

The clinical approach of the first two batches proved that the establishment of a non-specific symptomatic therapy is judicious, which, in addition to the failure to correct the disease, risks creating favorable conditions for the creation of chemotherapy resistance or antibiotic resistance. The result being an unsatisfactory one, which brings unjustified expenses to the operation.

Diagnosing the etiological agent through paraclinical examinations, in a specialized laboratory, provides clear and certain information, however, the average term of 5-10 days imposed for receiving the result, helps us to form an idea of the characteristics of the microbiome of the farm more than to quickly establish an etiologically related treatment.

On the other hand, the diagnosis by the method of quick antigen coupling tests from feces, is a simple, pragmatic and easy method to perform in the field.

With a high sensitivity and sensitivity, it shortens the response time and helps to establish an optimized treatment scheme in correlation with the etiological agent (20).

Within batch 7 - Paromomycin and Halofuginone, they proved similarities in treatment, however, by administering only 7 days of halofuginone compared to 11 of

paromomycin, makes halofuginone preferred in single Cryptosporidium parvum infestations. Besides this aspect, from the perspective of the broad spectrum of paromomycin, it must be used rationally to avoid antibiotic resistance.

The study showed good recovery in lambs treated with doses of 100 mg/kg of paromomycin, double the therapeutic indications on the Gabrovet leaflet. Toxicity reactions were not noted, even in the cases of severely dehydrated individuals.

The use of the non-steroidal anti-inflammatory, flunixin in a dose of 1-2mg/kg in young sheep, regardless of the causative etiological agent, proved the generation of a state of analgesia, the fight against colic and the reduction of the amount of faecal matter eliminated.

A peculiarity encountered at the farm level was the frequency of the discovery of abscesses in the thigh muscles, the area of choice for the administration of treatments, during the utilization of animals by slaughtering them, so molecules that support enteral administration were searched for in order to optimize the treatment.

Conclusions

The approach of a correlated etiological treatment, after the identification of the primary responsible pathogen agent, and the implementation of a modular therapy, individually adapted, allowed the reduction of mortality of young sheep which present peri- and neonatal diarrhea syndrome.

The diagnosis of the etiological agent, through rapid antigen fixation tests, allowed us to identify the biotic causes and allowed the early institution of treatment (compared to the usual methods which - classic bacteriological examinations that last at least 3-5 days).

The results obtained from our studies have shown that the dose of 100mg/kg of paromomycin (commercial product Gabrovet/CEVA) is also effective for combating *Cryptosporidium parvum* (compared to the use of the classical therapeutic dose of 50mg/kg, recommended by the manufacturer for combating *E. coli* infections) did not generate toxicity phenomena (evident through clinical examinations).

Curative use of Halofuginone has demonstrated similar efficacy to paromomycin in single *Cryptoporidium parvum* infestations.

The use of paromomycin has demonstrated a superior curative efficiency and therapeutic comfort compared to parenterally administered substances, because injectable (intramuscular) treatments in farm conditions have generated abscesses in the muscle mass, responsible for carcass deterioration.

References

1. **Aspinall V., Cappello M.,** Introduction of veterinary anatomy and physiology textbook, Butterworth Heinemann, Edinburgh, New York, 2004.
2. **Aydogdu, U., Isik N., Ekici, O.D., Yildiz, R., Sen, I., Coskun, A.,** Comparison of the Effectiveness of Halofuginone Lactate and Paromomycin in the Treatment of Calves Naturally Infected with Cryptosporidium parvum, Acta Scientiae Veterinariae, 2018, 46, 1, 9.
3. **Aloisio, F., Filippini, G., Antenucci, P., Lepri, E., Pezzotti, G., Caccio, S.M., Pozio, E.,** Severe weight loss in lambs infected with Giardia duodenalis assemblage B, Veterinary Parasitology, 2006, 142, 1-2, 154-8.
4. **Bacha, W.J., Bacha, L.M.,** Color Atlas of Veterinary Histology, 3rd Edition, Wiley Blackwell Publishing. New Jersey, SUA, 2012.
5. **Catchpole, J., Norton, C.C., Gregory, M.W.,** Immunisation of lambs against coccidiosis, Veterinary Records, 1993, 132, 3, 56-9.
6. **Codreanu, I.,** Textbook of veterinary physiology, Ed. Printech, București, 2018.
7. **Codreanu, M.,** Patologia medicală a animalelor domestice. Bolile aparatului urinar, Ed. Ex Terra Aurum, București, 2020.
8. **Codreanu, M.D.,** Terapeutică veterinară, Ed. Printech, Bucureşti, 2016.
9. **Constable, P.D., Hinchcliff, W.K., Done, H.S., Grünberg, W.,** Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats, Eleventh edition, Elsevier, Missouri, 2017.
10. **Dybreuil, J.D., Isaacson, R.E., Schifferli, D.M.,** Animal Enterotoxigenic Escherichia coli, EcoSal Plus, 2016, 7, 1.
11. **Foster, D.M., Smith, G.W.,** Pathophysiology of diarrhea in calves, Veterinary Clinics of North America: Food Animal Practice. 2009, 25, 13-36.
12. **Gyles, C.l., Fairbrother, J.M.,** Pathogenesis of Bacterial Infections in Animals, 5th Edition, Wiley-Blackwell; New York, 2022.
13. **Hodgson, J.C.,** Escherichia coli in domestic animals and humans, Wallingford, UK, 1994.
14. **Martella, V., Decaro, N., Buonavoglia, C.,** Enteric viral infections in lambs or kids, Veterinary Microbiology, 2015, 181, 1-2, 154 160.
15. **Mitrea, I.L., Ioniță, M.,** Diagnosticul protozoozelor la animale, Ed. Ceres, București, 2013.
16. **Mitrea, I.L.,** Parazitologie și boli parazitare, Ed. Ceres, București, 2011.
17. **Pugh, D.G., Baird, A.N., Edmondson, M., Passler, T.,** Sheep, Goat and Cervid Medicine, third edition, Elsevier, Missouri, 2021.
18. **Romero-Salas, D., Alvarado-Esquivel, C., Cruz-Romero, A., Aguilar-Dominguez, M., Ibarra-Piergo, N., Merino-Charezz, J.O., Perez De Leon, A.A., Hernandez-Tinoco, J.,** Prevalence of Cryptosporidium in small ruminants from Veracruz, Mexico, BMC Veterinary Research, 2016, 12, 14.

19. **Taylor, M.A., Bartman, D.J.,** The history of decoquinate in the control of coccidial infections in ruminants, Journal of Veterinary Pharmacology and Therapeutics, 2012, 35, 5, 417-427.
20. **Viu, M., Quilez, J., Sanchez-Acedo, C., Del Cacho, E., Lopez-Bernad, F.,** Field trial on the therapeutic efficacy of paromomycin on natural Cryptosporidium parvum infections in lambs, Veterinary Parasitology, 2000, 90, 3, 163-70.

IMPROVING THE PERFORMANCE OF PIGLETS

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Summary

In this paper, we wanted to present the results of the analysis of the reproductive performance of 400 sows found in maternity, at which had been reported deaths until the 28th-day p.b (postbirth), of 27%, unlike 16-18% that was expected. In this study, 90 sows were observed, 9 sows/day, and their offspring were weighed at birth, 24 hours postbirth and 28th day. Because of the nature of the technological flux, it was not the preferred method of analysis. In the end, the experiment was carried out over 11 days, the motive being the incapacity of weighing the piglets on the 28th day. For the experiment to be carried out properly, the subjects were separated into 5 groups: the first group represents the category of piglets of 100g or below, the second 100g-150g, the third 150-200g, the fourth 200-250g, and the last one the piglets that died in less than 24 hours. A correlation between the weight at birth, the quantity of the colostrum sucked, the weight at weaning and death can be seen after the analysis of the data was made.

Keywords: sow, reproduction, piglets, weigh.

Milk is essential for the growth, development and health of newborns. In some species of animals (sows and cows), milk production is a limiting factor in terms of growth and survival before weaning of offspring. Colostrum is the first secretion from the mammary gland and is ejected for several days after parturition. Colostrum is characterized by high concentrations of immunoglobulins necessary for conferring passive immunity in support of the underdeveloped immune system of the newborn. Colostrum is the first to determine the profitability of swine production, which is primarily dependent on both the body mass of the sow and the number and size of the nipples, which will determine the number and size of piglets produced per year per sow. The pig is a model of animal widely used in studies of the biology of lactation. However, due to the inadequate feed intake of nursing sows, they do not reach the realization of the physiological requirements for maximum milk yield, thus reaching a catabolic state, in which their body reserves are mobilized to provide the nutrients and energy for milk production during lactation. Most studies have focused on the hormonal regulation of lactation; however, little attention was paid to the development of nutritional strategies, which would increase lactogenesis in sows for the growth of piglets (16).

Materials and methods

In the first stage, which aimed at the quantitative evaluation of milk, the suckling capacity expressed by the amount of colostrum ingested by piglets was determined. The amount of colostrum ingested by a piglet will influence all its economic results. The activity began by evaluating production indices at a hall of 400 sows for which mortality was 27% compared to 16 -18%. how much is expected of this sector. Because the follow-up, as an experiment, on such a group is cumbersome, even impossible, under production conditions, 90 sows were studied, nine per day, closely following these nests, at which at the time of calving each piglet, after providing assistance in calving (removal of the placenta, cutting of the umbilical cord, wiping, drying the piglet and checking the vital functions) the piglets were identified with the initial of the mother and the order in which they were born. After carrying out the identification, the piglet was weighed, a value that represents the body mass at calving. Then they were given to the sow to be fed. The whole experiment lasted 11 days, because one day, for technical reasons, the weighing could not be performed. All data are reported as averages of piglets in a category. The materials used to collect the milk were a sterile container and gloves. When collecting colostrum and milk samples, having a relatively large number of suckling animals (about 600 sows) and the fact that an animal, at breastfeeding (1-3 minutes) provides a small amount of milk from a nipple, we resorted to collective sampling, but it was taken into account that the animals were of the same category of production, respectively to be on the same day of lactation, because we know that depending on the day of lactation, the quality and quantity of milk also varies.

Results and discussions

When collecting colostrum from recently calving animals, no problems were encountered because their general condition is a lethargic one, they accept any change, and decubital positions; but instead, as time goes by, that relationship of the mother cub sets in, a phenomenon that does not allow the collection of milk at any time, so for the weaning the sows were more and more vigilant, and the collection of milk samples was very difficult. To make it easier to visualize we divided the piglets by the amount of colostrum ingested in the first 24 hours as follows: piglets which, 24 hours after farrowing, had less than 0.675 g, up to the weight of farrowing (0 g); between 0 g and 100; between 100 g and 150 g; between 150 g and 200 g; between 200 g and 250 g. dead piglets at 24 hours (Fig. 1).

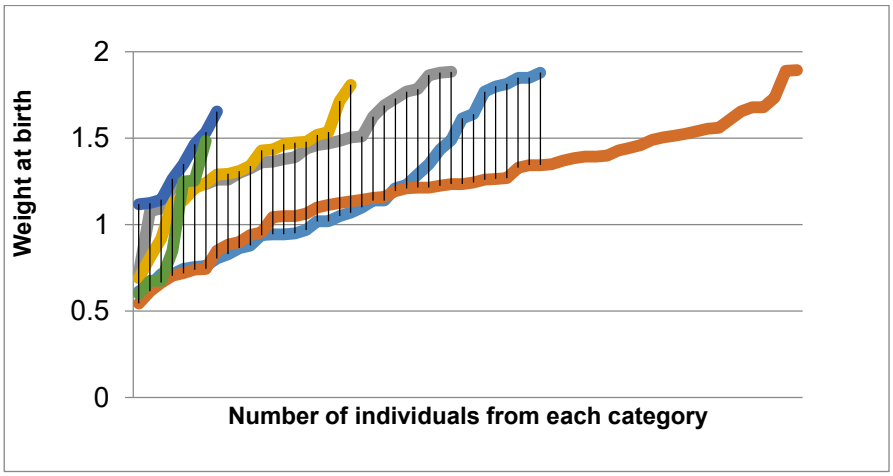

Fig. 1 Correlation between weaning weight and calving weight

We noticed that there is a correlation between all three values and as the weight of the piglet at calving is higher, both the amount of colostrum ingested as well as the weight when weaning increases proportionally. It was noticed that most of the piglets that were weaned below four kilograms were piglets that failed to assimilate from colostrum. Analyzing the situation at the hall level we can see that out of the total number of 6800 piglets calving, from the 400 sows, taken under study, 1828 piglets died until weaning and 4973 piglets were weaned, and the values of the weights obtained at weaning are as follows:

- 213 piglets had between 2.5 and 3 kg
- 893 piglets had between 3 and 4 kg
- 1530 piglets had between 4 and 5 kg
- 1360 piglets had between 5 and 6 kg
- 680 piglets had between 6 and 7 kg
- 298 piglets had over 7 kg.

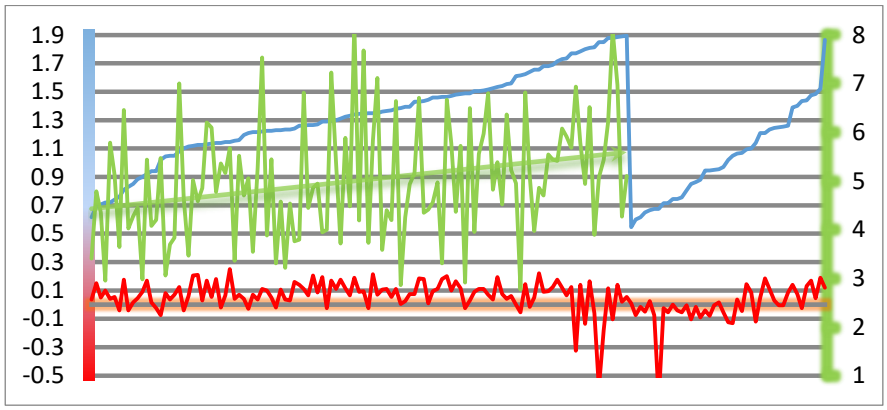

Fig. 2. Correlation between calving weight, amount of colostrum suckled and weaning weight

Conclusions

As a general conclusion we can say that ensuring a minimum amount of at least 100 grams of colostrum (recommended 200 grams) in the first 24 hours of life has a representative impact on the development of organisms, their state of health, resistance to diseases and many other consequences. Milk is of utmost importance for the survival, proper development and dynamic growth of the newborn. In addition, lactation is an integral component of the highly successful reproductive strategy of mammalian species. In fact, the development of the mammary gland, which is affected by the supply of AA, is closely related to the reproductive cycle of all mammalian species.

Acknowledgements

This work appeared within the project: Program for increasing performance and innovation in doctoral and postdoctoral excellence research - PROINVENT, Contract no. 62487/03.06.2022 POCU/993/6/13 - SMIS code: 153299.

References

1. **Dahanayaka, S., Rezaei, R., Porter, W.W., Johnson, G.A., Burghardt, R.C., Bazer, F.W., Hou, Y.Q., Wu, Z.L., Wu, G.,** Technical note: Isolation and characterization of porcine mammary epithelial cells, Journal of Animal Science, 2015, 93, 5186-93.
2. **Gallagher, D.P., Cotter, P.F., Mulvihill, D.M.,** Porcine milk proteins: A review, International Dairy Journal, 1997, 7, 99-118.
3. **Hurley, W.L.,** Mammary gland growth in the lactating sow, Livestock Production Science, 2001, 70, 149-57.

4. **Jenness, R., Holt, C.,** Casein and lactose concentrations in milk of 31 species are negatively correlated, Experientia, 1987, 43, 1015-8.
5. **Jenness, R.,** Biosynthesis and compounds of milk, Journal of Investigative Dermatology, 1974, 63, 109-18.
6. **Manjarin, R., Bequette, B.J., Wu, G., Trottier, N.L.,** Linking our understanding of mammary gland metabolism to amino acid nutrition, Amino Acids, 2014, 46, 2447-62.
7. **Mateo, R.D., Wu, G., Moon, H.K., Carroll, J.A., Kim, S.W.,** Effects of dietary arginine supplementation during gestation and lactation on the performance of lactating primiparous sows and nursing piglets, Journal of Animal Science, 2008, 86, 827.
8. **Quiniou, N., Dagorn, J., Gaudre, D.,** Variation of piglets' birth weight and consequences on subsequent performance, Livestock Production Science, 2002, 78, 63-70.
9. **Rezaei, R., Knabe, D.A., Li, X.L., Feng, S., Wu, G.,** Enhanced efficiency of milk utilization for growth in surviving low-birth-weight piglets, Journal of Animal Science Biotechnolgy, 2011, 2, 73-83.
10. **Shennan, D., Peaker, M.,** Transport of milk constituents by the mammary gland, Physiological Reviews, 2000, 80, 925-51.
11. **Sun, Y.L., Wu, Z.L., Li, W., Zhang, C., Sun, K.J., Ji, Y., Wang, B., Jiao, N., He, B.B., Wang, W.W., Dai, Z.L., Wu, G.,** Dietary L-leucine supplementation enhances intestinal development in suckling piglets, Amino Acids, 2015, 47, 1517-25.
12. **Trottier, N.L., Shipley, C.F., Easter, R.A.,** Plasma amino acid uptake by the mammary gland of the lactating sow, Journal of Animal Science, 1997, 75, 1266-78.
13. **Wolter, B.F., Ellis, M., Corrigan, B.P., Dedecker, J.M.,** The effect of birth weight and feeding supplemental milk replacer to piglets during lactation on preweaning and postweaning growth performance and carcass characteristics, Journal of Animal Science, 2002, 80, 301-8.
14. **Wang, W.W., Dai, Z.L., Wu, Z.L., Lin, G., Jia, S.C., Hu, S.D., Dahanayaka, S., Wu, G.,** Glycine is a nutritionally essential amino acid for maximal growth of milk-fed young pigs, Amino Acids, 2014, 46, 2037-45.
15. **Wu, G., Knabe, D.A., Kim, S.W.,** Arginine nutrition in neonatal pigs, Journal of Nutrition, 2004, 134, 2783S-90.
16. **Lei, J., Feng, D., Zhang, Y., Zhao, F.Q., Wu, Z., San, Gabriel, A., Fujishima, Y., Uneyama, H., Wu, G.,** Nutritional and regulatory role of branched-chain amino acids in lactation, Frontiers in Bioscience, 2012, 17, 2725-39.
17. **Wu, G., Bazer, F.W., Johnson, G.A., Knabe, D.A., Burghardt, R.C., Spencer, T.E., Li, X., Wang, J.,** Important roles for L-glutamine in swine nutrition and production, Journal of Animal Science, 2011, 89, 2017-30.

18. **Boyd, D.R., Kensinger, R.S., Harrell, R.J., Bauman, D.E.,** Nutrient uptake and endocrine regulation of milk synthesis by mammary tissue of lactating sows, Journal of Animal Science, 1995, 73, 36-56.
19. **Kim, S.W., Wu, G.,** Regulatory role for amino acids in mammary gland growth and milk synthesis, Amino Acids, 2009, 37, 89-95.
20. **Hurley, W.L.,** Mammary gland growth in the lactating sow, Livestock Production Science, 2001, 70, 149-57.

IN VITRO/VIVO PRODUCTION OF BOVINE EMBRYOS

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Summary

The term *in vitro fertilization* (IVF) suggests the fact that the union of the gametes is carried on outside the body, in a special environment under the control of successive stages, the result being of embryos. *In vitro embryo production* (IVP) is a technique that includes a combination of oocyte maturation, sperm capacitation, oocyte fertilization and in vitro embryo culture. IVP significantly contributes to increasing reproductive efficiency as a tool for shortening the interval between generations, intensifying selection, improving genetic material of economic interest. More than one million bovine embryos obtained through IVP were registered in 2018, representing 68.7% of the total. Among the 43 countries that reported ET-related data, 29 (67.4%) reported the use of *in vitro embryo production* (IVP). Since 2016, the production of embryos obtained in vitro (IVP), as an embryo generating technique, exceeded the number of embryos obtained in vivo (IVD) by 5.3%. In 2017, approximately 992,289 embryos were transferred following IVF and approximately 495,054 through IVD which indicates an approximately two-fold increase in favor of IVF. This increase in the number of embryos obtained in vitro is given by the increase of 164.7% in Europe and 82.7% in the USA, compared to 2016. Between 2007-2016, 605,550 embryos were obtained by IVD and 751,044 embryos were obtained through IVP. In Europe, in 2016, 19,974 IVFs were obtained and in 2017 a number of 52,879, representing a major increase in the use of this embryo production technique. Of the total embryos obtained in 2017 in Europe, Russia obtained 26,762, representing 51.5% of the total in Europe. In Romania, no data were reported regarding the production of embryos by this technique during the study period.

Keywords: embryo production, in vitro.

Bovine embryo transfer is used worldwide as a basis for genomic selection to improve livestock productivity (7, 15). Therefore, the embryos used in this process can be produced either in vivo or in vitro.

Although the in vitro culture conditions have been optimized over the years, they do not perfectly mimic the oviduct environment. Therefore, it is necessary for embryos to compensate for this by activating survival processes, such as the response to oxidative stress and changes in cellular metabolism (2, 16).

Furthermore, once transferred into the uterus of synchronized recipient females, not all embryos reach term. Studies show that 40% are lost between days 8 and 17 (9), so that approximately 60% survive until day 17, when the pregnancy is recognized by the mother through the secretion of IFN-T [1, 12] a cytokine whose expression is temporally and spatially regulated (5, 13, 14).

The cumulus cells surrounding the oocytes are able to secrete many cytokines and growth factors, crucial in oocyte nutrition and for embryo division subsequent fertilization. Growth factors and cytokines secreted by the cumulus cells are used to improve the potential of the implantation process (3).

Huang et al. reported that the IL (interleukine) system is an important factor in the embryo-maternal molecular communication during the implantation process. Glycosaminoglycans, cytokines, steroid hormones, growth factors and other nutrients secreted by the cumulus cells are important in nourishing the oocyte at various stages of nuclear and cytoplasmic maturation and subsequent development (20).

Calder et al. (3) studied oocyte development according to the four categories and observed that only category 4 oocytes showed reduced growth capacity. Oocytes from categories 1, 2, and 3 demonstrated similar developmental capacity for IVM.

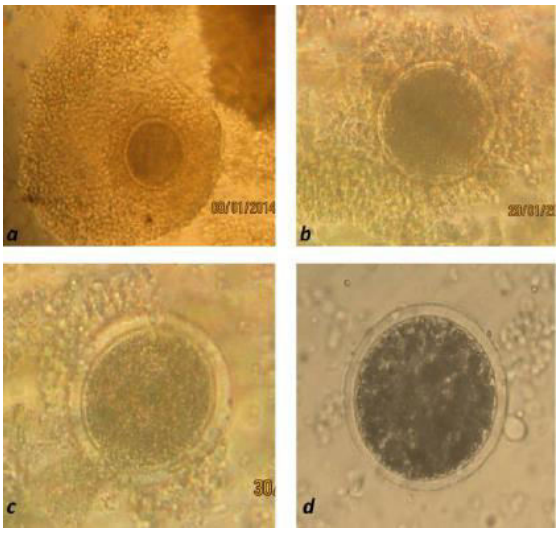

Fig. 1. Oocyte category I (a), oocyte category II (b), oocyte category III (c), Oocyte category IV (d) (3)

Advantages of in vitro fertilization

Through this technique, genetic improvement of the bovine herd is achieved in a shorter time, reducing the period between generations, by using high-yielding females and semen from bulls confirmed for the productions and expressed genetics. It also results in a higher number of embryos per donor, as follicular aspiration can be performed at short intervals. It can also be used in pregnant cows or shortly after calving (4).

IVF is indicated in high yielding females that are not suitable for a conventional ET process due to anatomical changes that prevent fertilization or metabolically changes that prevent fertilization or embryo retrieval. It is also indicated in females that have been removed from breeding due to conditions that do not alter ovarian function, such as metritis, post-surgical complications or limb injuries. IVP allows and facilitates the use of sexed sperm to obtain females with 90% efficiency (6).

It also allows obtaining offspring from females of high genetic quality that have to be slaughtered due to infectious diseases (tuberculosis, brucellosis, leucosis) (18).

In vitro fertilization (IVF) reduces the interval between generations, obtaining calves through sexed semen and expanding reproductive potential, perpetuating endangered cattle breeds, producing a large number of twins, pronuclear oocytes for DNA microinjection, cytoplasmic transfer, nuclear transfer, cloning by blastomeric recovery, conservation of endangered mammalian species (11).

Disadvantages of in vitro fertilization

Embryos produced in vitro are of poorer quality than those obtained in vivo and there are many differences between them. They usually have a smaller number of blastomeres, especially in the inner cell mass, a more fragile zona pellucida, a reduction in the perivitelline space, and a faster rate of development (11).

They also display changes in intercellular communication, with an abnormal expression of proteins that form Gap junctions, high triglycerides and lower other lipids, changes in gene expression, a higher incidence of apoptosis (17).

In addition, embryos produced in vitro have a lower resistance to cryopreservation, which is one of the main problems, because the survival after cryopreservation is lower compared to that of embryos produced in vivo, which makes their long-term preservation difficult (10).

Statistics

Since 2016, the production of embryos obtained in vitro exceeded the number of embryos in vivo by 5.3%. In 2017, approximately 992,289 embryos were transferred through IVP, and approximately 495,054 through IVD (table 1), thus indicating an approximately two-fold increase in favor of IVP. Compared to 2016, this increase in the number of embryos obtained in vitro is due to the increase of 164.7% in Europe and 82.7% in the USA. Between 2007-2016, 605,550 embryos were obtained through IVD and 751,044 embryos through IVP (see Table 1).

In Europe, in 2016, 19,974 IVFs were obtained, and in 2017 a number of 52,879, which indicates a major increase in the use of this embryo production technique. Of the total embryos obtained in 2017 in Europe, Russia obtained 26,762, representing 51.5% of the total in Europe. In Romania, no data were reported regarding the production of embryos by this technique during the study period (18).

Table 1

Total embryos transferred by IVD and IVP in 2017 in bovines, horses, sheep, goats in different regions of the globe (18)

Regions	Bovines		Horses		Sheep		Goats	
	IVD	IVP	IVD	IVP	IVD	IVP	IVD	IVP
Africa	5.126	5.423	0	0	127	0	0	0
Asia	212	0	0	0	0	0	0	0
Europa	143.246	52.879	1.211	1.142	6.435	0	76	0
North America	292.755	475.969	50	0	2.826	66	3.106	61
Oceania	4.485	4.332	0	0	0	0	0	0
South America	49.230	453.686	19.560	180	9.264	0	793	0
Total	**495.054**	**992.289**	**20.821**	**1.322**	**18.652**	**66**	**3.975**	**61**

Bovines are the species most used in embryo collection techniques with 1,499,367 embryos collected or produced in 2018 (96.7% - IETS Report, 2019). There was a relative stabilization in this species with regard to ET activities compared to 2017 (+ 0.8%), in contrast to an increase of 23.4% compared to the period 2014-2017. The main trends identified in 2017 were observed again in 2018 (19).

Table 2

Total embryos transferred by IVD and IVP in 2018 in bovines, horses, sheep and goats in relation to different regions (18)

Regions	Bovines		Horses		Sheep		Goats	
	IVD	IVP	IVD	IVP	IVD	IVP	IVD	IVP
Africa	6.651	3.741	0	0	3.325	0	100	660
Asia	162	0	0	0	0	0	0	0
Europa	141.209	61.816	1.395	2.107	3.661	457	227	158
North America	270.187	503.718	1.149	0	2.158	0	8.075	0
Oceania	4.445	11.997	0	0	2.970	0	0	0
South America	47.313	448.128	18.768	346	5.239	55	402	0
Total	**469.967**	**1.029.400**	**21.312**	**2.453**	**17.353**	**512**	**8.804**	**818**

Thus, a decrease (-5.1%) in the number of embryos obtained in vivo (IVD) and an increase (+ 2.6%) in the production of embryos were reported IVP. More than one million bovine embryos obtained through IVP were registered in 2018, representing 68.7% of the total. Among the 43 countries that reported ET-related

data, 29 of them (67.4%) reported the use of IVP. Although this technology was the main source of embryos in only 18 countries, they accounted for 85.7% of all embryos produced worldwide (19) (see Table 2).

Conclusions

Although well established, IVP techniques support further development, IVF representing the highlight of nowadays use.

The full potential of IVF was not achieved, as shown by statistics data which emphasize a visible discrepancy between countries.

References

1. **Bazer, F.W., Burghardt, R.C., Johnson, G.A., Spencer, T.E., Wu, G.,** Mechanisms for the establishment and maintenance of pregnancy: synergies from scientific collaborations, Biology of Reproduction, 2018, 99, 225-241.
2. **Bertoldo, M.J., Locatelli, Y., O'Neill, C., Mermillod, P.,** Impacts of and interactions between environmental stress and epigenetic programming during early embryo development, Reproduction, Fertility, and Development, 2015, 27, 1125-1136.
3. **Calder, M.D., Caveney, A.N., Smith, L.C., Watson, A.J.,** Responsiveness of bovine cumulus-oocyte-complexes (COC) to porcine and recombinant human FSH, and the effect of COC quality on gonadotropin receptor and Cx43 marker gene mRNAs during maturation in vitro, Reproductive Biology and Endocrinology, 2003, 1, 14.
4. **Cihangir, N., Görkemli, H., Özdemir, S., Aktan, M., Duman, S.,** Influence of cumulus cell coculture and cumulusaided embryo transfer on embryonic development and pregnancy rates, Journal of the Turkish-German Gynecological Association, 2010, 11, 121-126.
5. **Ealy, A.D., Yang, Q.E.,** Control of interferon-tau expression during early pregnancy in ruminants, American Journal of Reproductive Immunology, 2009, 61, 95-106.
6. **Ferré, L.B., Kjelland, M.E., Strøbech, L.B., Hyttel, P., Mermillod, P., Ross, P.J.,** Review: Recent advances in bovine in vitro embryo production: reproductive biotechnology history and methods, Animal, 2020, 14, 5, 991-1004.
7. **Hasler, J.F.,** Forty years of embryo transfer in cattle: a review focusing on the journal theriogenology, the growth of the industry in North America and personal reminisces, Theriogenology, 2014, 81,152-169.
8. **Kouamo, J., Dawaye, S.M., Zoli, A.P., Bah, G.S.,** Evaluation of bovine(Bos indicus) ovarian potential for in vitro embryo production in the Adamawa plateau (Cameroon), Open Veterinary Journal, 2014, 4, 2,128-136.
9. **Mamo, S., Mehta, J.P., McGettigan, P., Fair, T., Spencer, T.E., Bazer, F.W., Lonergan, P.,** RNA sequencing reveals novel gene clusters in bovine

conceptuses associated with maternal recognition of pregnancy and implantation, Biology of Reproduction, 2011, 85, 1143-1151.

10. **Molina, I., Cervera, R., Duque, C., Alfonso, J., Romeu, A.,** Criopreservación de ovocitos humanos. Vitrifcación vs. Congelación, Rev Iber Fert, 2004, 21, 3, 177-189.
11. **Rajesh, W., Subha, G., Parveez, A.P., Praveen, K.P., Kausar, Q.,** Relevance and Technological Implications of in Vitro Fertilization in Animal Breeding and Reproduction: A Review, International Journal of Emerging Technology and Advanced Engineering, 2015, 13, 255-258.
12. **Randi, F., Fernandez-Fuertes, B., McDonald, M., Forde, N., Kelly, AK., Bastos Amorin, H., Muniz de Lima, E., Morotti, F., Marcondes Seneda, M., Lonergan, P.,** Asynchronous embryo transfer as a tool to understand embryo–uterine interaction in cattle: is a large conceptus a good thing?, Reproduction, Fertility and Development, 2016, 28, 1999-2006.
13. **Sakurai, T., Bai, H., Bai, R., Sato, D., Arai, M., Okuda, K., Ideta, A., Aoyagi, Y., Godkin, J.D., Imakawa, K.,** Down-regulation of interferon tau gene transcription with a transcription factor, EOMES, Molecular Reproduction and Development, 2013, 80, 371-383.
14. **Sakurai, T., Sakamoto, A., Muroi, Y., Bai, H., Nagaoka, K., Tamura, K., Takahashi, T., Hashizume, K., Sakatani, M., Takahashi, M., Godkin, J.D., Imakawa, K.,** Induction of endogenous interferon tau gene transcription by CDX2 and high acetylation in bovine nontrophoblast cells, Biology of Reproduction, 2009, 80, 1223-1231.
15. **Stroebech, L., Mazzoni, G., Pedersen, H.S., Freude, K.K., Kadarmideen, H., Callesen, H., Hyttel, P.,** In vitro production of bovine embryos: revisiting oocyte development and application of systems biology, Animal Reproduction, 2015, 12, 465-472.
16. **Thompson, J.G., Mitchell, M., Kind, K.L.,** Embryo culture and long-term consequences, Reproduction, Fertility and Development, 2007,19,43-52.
17. **Vajta, G.,** Vitrifcation of the oocytes and embryos of domestic animals, Animal Reproduction Science, 2000, 60-61, 357-64.
18. **Viana, J.,** Embryo industry on a new level: over one million embryos produced *in vitro*, Embryo Technology Newsletter, 2019, 36, 4.
19. **Wrenzycki, C.,** In vitro culture systems: how far are we from optimal conditions?, Animal Reproduction, 2016, 13, 279-282.
20. **Huang, X., Zhang, C.H., Wang, Z.B., Quan, S., Tong, J.S., Ma J.Y., Guo, L., Wei, Y.C., Ouyang, Y.C., Hou, Y., Xing, F.Q., Sun, Q.Y.,** GM130, a cis-Golgi protein, regulates meiotic spindle assembly and asymmetric division in mouse oocyte, Cell Cycle, 2011, 10, 1861-1870.

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