**UNIVERSITATEA DE ŞTIINŢE AGRICOLE
ŞI MEDICINĂ VETERINARĂ A BANATULUI
TIMIŞOARA**

FACULTATEA DE MEDICINĂ VETERINARĂ

LUCRĂRI ŞTIINŢIFICE

**MEDICINĂ VETERINARĂ
TIMIŞOARA
VOLUMUL LV (2)**

**SCIENTIFICAL PAPERS
VETERINARY MEDICINE**

To be cited: LUCRARI STIINTIFICE: MEDICINA VETERINARA TIMISOARA (SCIENTIFICAL PAPERS: VETERINARY MEDICINE TIMISOARA)**,** vol. LV (2), 2022

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

**UNIVERSITATEA DE ŞTIINŢE AGRICOLE
ŞI MEDICINĂ VETERINARĂ A BANATULUI
TIMIŞOARA**

FACULTATEA DE MEDICINĂ VETERINARĂ

LUCRĂRI ŞTIINŢIFICE

**MEDICINĂ VETERINARĂ
TIMIŞOARA
VOLUMUL LV (2)**

**SCIENTIFICAL PAPERS
VETERINARY MEDICINE**

**TIMIŞOARA
2022**

DISPOSAL OF ANTIMICROBIALS BY POULTRY AND FISH FARMERS IN ZARIA METROPOLIS, KADUNA STATE, NIGERIA

ALIYU M.B.[1], BUCUR I.[2], ADIKWU A.[1], TIRZIU E.[2], IBRAHIM N.B.[1], OBIȘTIOIU D.[2], OKOLOCHA E.C.[1]

[1]Ahmadu Bello University, Faculty of Veterinary Medicine
810107, Sokoto Road, Samaru, Zaria, Kaduna State, Nigeria
[2]Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timișoara, Faculty of Veterinary Medicine, 300645,
Calea Aradului No. 119, Timișoara, Romania
E-mail: mailaliyu@gmail.com

Summary

Poultry and aquaculture are the fastest-growing animal food-producing sector where the practices include the use of antimicrobial for disease treatment, prevention, and growth promotion. The indiscriminate use of antimicrobials by farmers has resulted in the proliferation of higher concentrations of antimicrobials in the environment ('Antimicrobial pollution') through contamination of Animals' waste streams. This is mainly because these administered antimicrobials are not fully metabolized, therefore released unchanged into the environment. Due to the lack of information on antimicrobial disposals in the Zaria metropolis, this study surveyed the ways antimicrobials are disposed of by 200 fish and poultry farmers and their awareness level of the environmental effects. The major methods of antimicrobial disposal were the refuse dump – disposal 40 (20%), irrigation water 40 (20%), and wastewater 120 (60%). There was a very poor (70%) level of "antimicrobial pollution" awareness among the farmers, hence the need to sensitize them and develop a proper disposal approach with the Ministries of Agriculture and Environment.

Keywords: disposal, antimicrobials, poultry, aquaculture, pollution.

Poultry and fish farming have been intensified globally to meet the high protein demand, hence the increased usage of antimicrobials in these sectors. The improper usage and disposal contribute to the spreading and occurrence of antimicrobial resistance (10). However, the antimicrobial consumption profiles in developing countries are greatly influenced by the gross abuse and misuse of these antimicrobial agents due to their availability over the counter, through unregulated supply chains, and the purchase without prescriptions (2). Antimicrobial resistance remains a growing threat to human and animal health, lessening the ability to treat bacterial infections and furthering the risk associated with morbidity and mortality caused by resistant bacteria (8).

Diverse means of antimicrobials released into the environment include human waste streams and wastes from veterinary use and livestock farming (7). Antimicrobials used for prophylaxis or therapy in humans contaminate the human waste streams, likewise, the antibiotics used in animals including poultry and fish for growth promotion, prevention, and treatment equally contaminate the animals' waste streams. Thus, these are considered prime sources of antimicrobial disposal in the

environment (5). This is mainly because these administered antimicrobials are not fully metabolized, therefore released unchanged into the environment, i.e., water, manure, or soils. Nevertheless, these waste streams will contain both antimicrobials and resistance genes; both are considered pollutants (16). The contribution of resistant microbes from various sources seems to be the major base of resistance in the environment (10). Sub-Saharan Africa and South Asia have the highest bacteria drug-resistant and antimicrobial pollution of the environment (15). The area of study falls under this region of significant concerns. This study aims to determine the ways antimicrobials are disposed of by poultry and fish farmers and awareness of the effects on the environment.

Materials and methods

The study was carried out in the Zaria metropolis which is made up of Sabon Gari and Zaria local government areas (LGA) in Kaduna State, Nigeria (11.0855° N, 7.7199° E). A cross-sectional study was carried out on a total of 200 farms comprising 140 poultry farms and sixty (60) fish farms (aquaculture) selected based on the convenience and consent of farm owners.

A structured close-ended questionnaire was administered via oral interview to selected farm owners. Seven questions were targeted at extracting the demography while twelve questions were set to determine the knowledge and practice/perception towards disposal of antimicrobial agents by poultry and fish farmers in the Zaria metropolis. The data obtained were collated and imputed in Microsoft® excel and represented in tables and graphs.

Results and discussions

The demographic characteristics of the farmers reveal that 137 (69%) were males, while 63 (31%) were females (Table 1). This is due to the cultural orientation of Northern Nigeria where fish and poultry farming is dominated by the male gender (11). The study area is an educational hub in the country, hence the majority (46%) have tertiary education. This is a positive factor, as it will allow for easy enlightenment and education of the farmers as regards antimicrobial disposal, since a good education level aid understanding of disease transmission (4). Generally, there are more poultry farms in Zaria, hence the coverage of 140 (70%) as compared to fish farms (30%). This is due to the low cost of production, ease of setup (9), and less water consumption needed for the establishment of a poultry farm.

This study established the fact that both fish and poultry farmers 123 (62%) are aware of the potential health hazards antimicrobial agents can pose to our environment. Although the majority of respondents from the poultry farms 106 (76%) showed some knowledge about antimicrobial resistance, a significant number of fish farm owners 49 (81%) did not know about it despite the supposed abundance of information and awareness about the health challenges associated with

antimicrobial resistance. This corroborated the study by Hedman et al. (8) in poultry from low-income countries and that of Okocha et al. (17) in aquaculture which all reported lack of awareness as the cause of indiscriminate use of antibiotics and hence breeding of antimicrobial resistance (Table 2).

Table 1

Demographic Characteristics of the Respondents to Disposal of Antimicrobials by Poultry and Fish Farmers in Zaria, n=200

Variables		Frequency	Percentage (%)
Sex	Male	137	69
	Female	63	31
Age	10 – 20 years	0	0
	21 – 30 years	37	18.5
	31 – 40 years	81	40.5
	41 – 50 years	40	20
	>50years	42	21
Education	None	0	0
	Primary	39	19.5
	Secondary	65	32.5
	Tertiary	96	48
Type of farm	Fish farm	60	30
	Poultry farm	140	70
Number of years in practice	0– 5 years	80	40
	5 – 10 years	96	48
	>10 years	24	12

This study also established that asides from the use of antimicrobials for curative purposes 156 (78%), the use of antibiotics for prophylactic purposes and the unpleasant practice of routine administration of antimicrobials still exists in farms in our communities. This practice plays a major role in the availability of excess antimicrobials constantly deposited into the environment from these farms. This finding again supports the already published works of Sekyere et al. (18) done pig farms in Ghana, FAO (6), and Manyi-Loh et al. (13) which all associated the contamination of the environment to prophylactic/routine use of antimicrobial agents by animal farms.

Table 2

Knowledge and Practices of Respondents on Antimicrobial Disposal by Poultry and Fish Farmers in Zaria, n=200

Questions	Fish farm	Response	Poultry farm	Response
Do you use antimicrobials	Yes	60	Yes	140
	No	0	No	0
Type of antimicrobial	Antifungal	12	Antifungal	0
	Antibiotics	48	Antibiotics	76
	Antiparasitic	0	Antiparasitic	28
	Antiviral	0	Antiviral	36
Do you know about antimicrobial resistance	Yes	11	Yes	106
	No	49	No	34
Do you think antimicrobials can cause harm to humans and animals	Yes	60	Yes	140
	No	0	No	0
Do you think antimicrobials can cause harm to the environment?	Yes	36	Yes	87
	No	24	No	53
How often do you use antimicrobials on your farm?	Prophylaxis	0	Prophylaxis	32
	Curative	60	Curative	96
	Routinely	0	Routinely	12
Who prescribes antimicrobial for the farm	Self-prescription	12	Self-prescription	17
	Veterinarian	24		74
	Others	24	Veterinarian	49
			Others	
What is the approximate duration of disposal after use?	1 – 6 hours	11	1 – 6 hours	0
	6 – 12 hours	35	6 – 12 hours	59
	12 – 24 hours	14	12 – 24 hours	81
Do you feel your method of antimicrobials disposal can cause harm to humans and animals?	Yes	9	Yes	2
	No	51	No	138
Do you feel your method of antimicrobials disposal can cause harm to the environment?	Yes	7	Yes	31
	No	53	No	109

Appropriate disposal of remnant and expired antimicrobial is of great importance because of the impending environmental hazards and public health risks.

The present study indicates that poultry and fish farms dispose of their remnant antimicrobial through refuse dump 40 (20%), wastewater 119 (59.5%), and irrigation water 39 (20%) (Fig. 1). The disposal method for expired antimicrobials was mainly through the refuse dump disposal method which was 75% and 91% for fish and poultry farms respectively (Fig. 2.). This finding is similar to a study in Anambra, South-East, Nigeria (14) which reported unused and expired antibiotics are disposed of by being thrown into the garbage, water fills, and sewers because of the costs associated with proper disposal of those medications. When not degraded or eliminated during wastewater treatment, disposed antimicrobials reach surface water or are passed into the aquatic environment. Recently, it was reported that the world's rivers are far more polluted with antimicrobial than anticipated, hence becoming a global threat to human health and the environment (3).

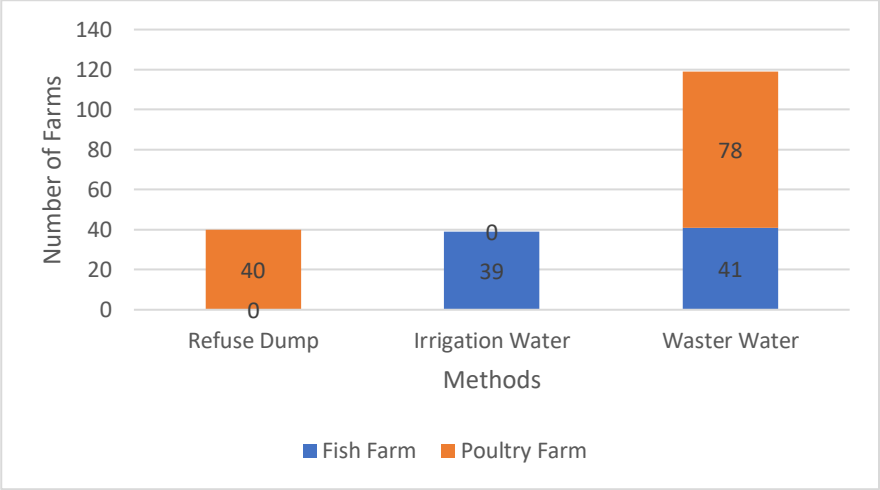


Fig. 1. Disposal Methods of Remnant Antimicrobial Administered at Poultry and Fish Farms

From this present study, the majority of the farmers 189 (94.5%) believe that their methods of disposal do not cause harm to animals (Table 2). This finding buttresses the results obtained in another study carried out in Ibadan, South-West, Nigeria (1) which reported that only a few farmers know the impact of waste disposal on the environment. Although the concentration of these disposed antimicrobial agents is negligible, such concentrations can promote resistance either by horizontal gene transfer or by modifying targets (19). The indiscriminate release of antimicrobials into the environment compromises the effectiveness of antibiotics and augments resistance as harmless microbes mutate to deadly and resistant pathogens (12). Consequently, when transmitted to humans, these bacteria are

already resistant to the available antibiotics, leading to increased morbidity and mortality and an increased economic burden to the health care system (20).

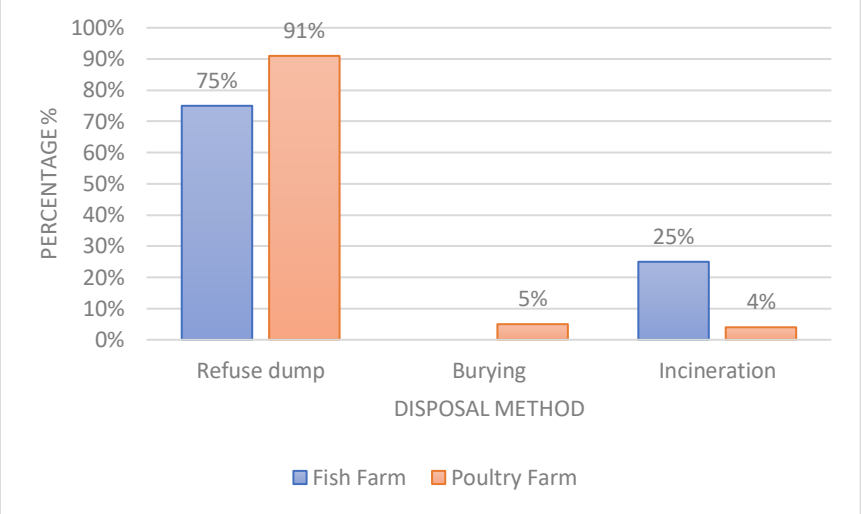


Fig. 2. Distribution of Expired Antimicrobial Disposal Methods Among Fish and Poultry Farm

Fig. 3. A fish farm where the remnant of antimicrobial is disposed as irrigation water

Fig. 4. A refuse dump disposal method for remnant and expired antimicrobial

Conclusions

Refuse dump, irrigation water, and wastewater were established to be the methods of antimicrobial disposal in poultry and fish farms with the wastewater method being the predominant method used in both farms. The farmers are generally unaware of the effects of discharging untreated antimicrobials into the environment.

A thorough assessment of disposal practices of antimicrobial among livestock, poultry, and fish farms is required to get a real-time picture of the severity of the issue. Addressing this problem will involve the need for adequate wastewater treatment and sewage connectivity to avoid environmental contamination. This process is very expensive, hence the need for collaboration among the ministry of Agriculture, Health, and Environment. The sustainability of the intervention will play a key role in the fight against antibiotic misuse and resistance.

References

1. **Adelowo, O.O., Okunlola, I.,** Field assessment of antibiotic use in fish farms in Southwestern Nigeria, Revue d'élevage et de médecine vétérinaire des pays tropicaux, 2019, 72, 4, 187-191.
2. **Ayukekbong, J.A., Ntemgwa, M., Atabe, A.N.,** The threat of antimicrobial resistance in developing countries: causes and control strategies, Antimicrobial Resistance and Infection Control, 2017, 6, 47- 51.
3. **Chang, Q., Wang, W., Regev-Yochay, G., Lipsitch, M., Hanage, W.P.,** Antibiotics in agriculture and the risk to human health: how worried should we be?, Evolutionary Applications, 2015, 8, 3, 240-247.
4. **Diaz-Quijano, F.A., Martínez-Vega, R.A., Rodriguez-Morales, A.J., Rojas-Calero, R.A., Luna-González, M.L., Díaz-Quijano, R.G.,** Association between the level of education and knowledge, attitudes and practices regarding dengue in the Caribbean region of Colombia, BMC Public Health, 2018, 18, 1, 143-152.
5. **Dolliver, H., Gupta, S.,** Antibiotic losses in leaching and surface runoff from manure-amended agricultural land, Journal of Environmental Quality, 2008, 37, 3, 1227-1237.
6. **FAO**, The State of World Fisheries and Aquaculture. Contributing to food security and nutrition for all, Rome: FAO, 2016.
7. **Gillings, M.R.,** Evolutionary consequences of antibiotic use for the resistome, mobilome, and microbial pangenome, Frontiers in Microbiology, 2013, 4, 4-10.
8. **Hedman, H.D., Vasco, K.A., Zhang, L.,** A Review of Antimicrobial Resistance in Poultry Farming within Low-Resource Settings, Animals, 2020, 10, 8, 1264-1270.
9. **Heise, H., Crisan, A., Theuvsen, L.,** The poultry market in Nigeria: market structures and potential for investment in the market, International Food Agribusiness Management Review, 2015, 18, 197-222.

10. **Ibrahim, M., Ahmad, F., Yaqub, B., Ramzan, A., Imran, A., Afzaal, M., Mirza, S.A., Mazhar, I., Younus, M., Akram, Q., Taseer, M.S.,** Current trends of antimicrobials used in food animals and aquaculture, antibiotics and antimicrobial resistance genes in the environment, Antibiotics and Antimicrobial Resistance Genes in the Environment, 2020, 39-69.
11. **Ladan, A.A., Okolocha, E.C., Kabir, J., Bolorunduro, P.,** Assessment of antimicrobial drugs use and their residue in the farmed fish of Kaduna, Nigeria, Global Journal of Fisheries Science, 2021, 3, 3,15-26.
12. **Larsson, D.G.,** Antibiotics in the environment, Upsala Journal of Medical Sciences, 2014, 119, 2, 108-112.
13. **Manyi-Loh, C., Mamphweli, S., Meyer, E., Okoh, A.,** Antibiotic Use in Agriculture and Its Consequential Resistance in Environmental Sources: Potential Public Health Implications, Molecules, 2018, 23, 4, 795-805.
14. **Michael, I., Ogbonna, B., Sunday, N., Anetoh**, **M., Matthew**, **O.,** Assessment of disposal practices of expired and unused medications among community pharmacies in Anambra State southeast Nigeria: a mixed study design, Journal of Pharmaceutical Policy and Practice, 2019, 12, 1, 12-22.
15. **Murray, C.J., Ikuta, K.S., Sharara, F., Swetschinski, L., Aguilar, G.R., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S.C.,** Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis, The Lancet, 2022, 399, 629 - 655.
16. **Nesme, J., Simonet, P.,** The soil resistome: a critical review on antibiotic resistance origins, ecology, and dissemination potential in telluric bacteria, Environmental Microbiology, 2015, 17, 4, 913-930.
17. **Okocha, R.C., Olatoye, I.O., Adedeji, O.B.,** Food safety impacts of antimicrobial use and their residues in aquaculture, Public Health Reviews, 2018, 39, 21-31.
18. **Sekyere, J.O.,** Antibiotic types and handling practices in disease management among pig farms in Ashanti region, Ghana, Journal of Veterinary Medicine, 2014, 42-50.
19. **Sultan, I., Rahman, S., Jan, A.T., Siddiqui, M.T., Mondal, A.H., Haq, Q.M.R.,** Antibiotics resistome and resistance mechanisms: A bacterial perspective, Frontier Microbiology, 2018, 9, 2066-2074.
20. **Wilkinson, J.L., Alistair, B.A., Boxall, D.W., Kolpin, K.M.Y., Leung, R.W.S., Lai, C.G., Aiko D.A.,** Pharmaceutical pollution of the world's rivers, Proceedings of the National Academy of Sciences, 2022, 119, 8-21.

SURGICAL MANAGEMENT AND POST-OPERATION EVOLUTION OF CUTANATE NEOPLASIES IN 12 DOGS

AȘTILEAN A.N., OBER C., PEȘTEAN C., TĂBĂRAN F., OANA L.

University of Agricultural Sciences and Veterinary Medicine,
Faculty of Veterinary Medicine, 400372,
Mănăștur Road nr. 3-5, Cluj-Napoca, România
E-mail: andreea-niculina.astilean@usamvcluj.ro

Summary

Between January 2019 and March 2021, 12 dogs presented for consultation within the Surgical Discipline of the Faculty of Veterinary Medicine, Cluj-Napoca, being diagnosed with various types of skin neoplasms. The tumor parameters observed at the time of their physical examination were: size, anatomical location, consistency, color, presence or absence of infiltration of neighboring tissues, adhesion to the underlying planes, skin-associated lesions and lymph node reactions. A presumptive diagnosis was elaborated through clinical, cytological, radiological evaluations, and the definite diagnosis was made postoperatively by histopathological examination within the discipline of Pathological Anatomy, FMV Cluj - Napoca. All patients received surgical treatment. The surgical technique was represented by the procedure of resection of tumor masses with safety margins specific to each tumor type and a fascial plane, with or without skin plastics. The dominant skin neoplasms were mast cell tumors (MCT) of varying degrees. The follow-up time of the evolution of postoperative clinical signs ranged from 3 to 12 months. The disease-free interval was established in 7 dogs, which was 7.4 months, the limits being between 5 and 12 months. Our results showed that the use of surgical technique for resecting tumor masses with safety margins specific to each tumor type and an underlying fascial plane is a viable treatment solution, even if a single treatment was used without complementary therapy (radiotherapy, chemotherapy).

Keywords: skin neoplasms, dog, surgical treatment, resection.

Skin and subcutaneous tumors in dogs are the most common tumors in canine oncological pathology, accounting for one-third of all tumors that occur in this species (1, 4, 14). Annual reported incidence estimates skin and subcutaneous tumors to be about 450.000 dogs (1, 15). There are many types of tumors that appear on the skin, one of the most common being mast cell tumor. About 20% to 40% of primary cutaneous and subcutaneous tumors, are histologically diagnosed as malignant in dogs (1, 2).

One study reported a calculation of the growth rate of malignant skin tumors in dogs by age and breed (8). These researchers found that the risk increased linearly by a factor of 1.1 per year with age, and pure breed dogs were twice as susceptible as common breeds to develop malignancies (20).

Occasionally, skin tumors in dogs appear as secondary metastatic lesions, and the clinician should consider this possibility, especially for his list of differential diagnoses (3). Skin tumors generally occur in older animals and there appears to be no significant differences regarding sex predisposition (7). Some of the differences

that exist are related to the predilection for certain breeds (20).

The etiology of skin tumors has only been proven for a few types of tumors. Although, their etiology is multifactorial and still unknown, current research will shed light on this topic. The factors that are most commonly incriminated in the oncogenesis of skin tumors are: physical factors (radiation, heat damage), genetic factors and molecular influence, hormonal, vaccines, viruses and immune influence (5, 16, 18).

The aim of this study was to describe the main skin tumors, the surgical technique, to evaluate the clinical result, the long-term effect and the complications that may occur on the skin function following the use of the surgical technique of excision of the neoplasms to monitor the median survival time (MST) and disease-free interval (DFI) following surgical excisions. In order to do this, the following specific objectives have been defined: collecting information from the clinical records of all animals undergoing the procedure of excision of the skin tumors performed in the Departament of Surgery in collaboration with the Department of Pathological Anatomy, between January 2019 - March 2021; description of skin tumors; description of the surgical procedure; clinical presentation of patients; identification of tumor formations and their description within the discipline of Pathological Anatomy - pre and postoperative; comparison of the sample and results with the literature; identification of complications and limitations associated with surgical technique; identification and assessment of median survival time and disease-free interval of tumor formations resulting from surgery; the impact of postoperative recovery on the life of the owner.

Materials and methods

All patients included in the present study on the long-term follow-up of clinical effects that may occur on the skin and other organs following metastases, were examined between 2019 and 2021 in the Department of Surgery, FMV Cluj-Napoca and received complete clinical examination, along with hematological, biochemical, cytological, histological examinations, but also lateral-lateral and ventro-dorsal radiographs of the thoracic cavity, which were performed for each of the 12 dogs, preoperatively. The data of each dog were recorded on the clinical examination sheets, and each owner gave his written consent to the procedures to which the animal was to be subjected, stating that he understood their risks. The appearance of skin tumors was remedied by surgical excision with safety margins specific to each tumor type. Signs of patients with major significance in terms of risk factors for skin tumors that have been recorded are: age, breed, sex, as well as information regarding previous treatments, the pathology's onset until diagnosis, and in addition to this, information on growth rate, local inflammatory signs, pruritus, change in appearance over time. The anamnesis focused on collecting information on general chronic or acute signs (changes in body weight, appetite, diet, intolerance to effort, cough, etc.), but also those of possible paraneoplastic syndromes (gastric problems,

polyuria, polydipsia, hypercalcemia). Tumor parameters size, anatomical location, consistency, color, presence or absence of infiltration of neighboring tissues, skin ulcers, inflammation were also examined. Exploratory lymph nodes were also evaluated during the consultation and were part of the standard procedure.

The diagnosis of skin tumors was made on the basis of detailed anamnesis, history, physical examination, laboratory examinations, cytological and histological examinations, radiographs and evaluation of lymph nodes both palpatively and cytologically. From the bioptic techniques proposed by the literature, we chose the fine needle biopsy performed preoperatively. In order to perform this technique, the tumor formation requires a mechanical grooming, trimming both the formation and around it and asepsis with chlorhexidine solution 4% and ethyl alcohol. The technique is based on the use of a fine 23 G needle, attached to a drawn plunger syringe, which will be inserted into the tumor mass and cells will be extracted by aspiration from the incriminated tissue. This content is expressed on a slide, previously degreased, which is fixed and colored (Diff-Quick). All patients admitted to the study received a cytological examination. All samples collected intraoperatively were preserved with 10% formic aldehyde solution in a ratio of 1:10 to the sample to be analyzed.

These were recorded and processed within the discipline of Pathological Anatomy, FMV Cluj-Napoca, after which an analysis bulletin was issued for each patient by the pathologist. For histopathologic examination, the tissues were fixed in 10% phosphate-buffered neutral formalin, routinely processed, paraffin embedded, and stained with hematoxylin and eosin (H&E). Replicate sections of particular cases were also stained with special stains such as Oil Red O, Giemsa, periodic acid-Schiff and toluidine blue whenever they were needed to confirm the diagnosis (13).

At the time of discharge from the veterinary hospital, all owners were asked to return with their dogs for clinical examination at 3, 6 and 12 months postoperatively, respectively, in order to observe the patient's progress based on the objective clinical examination and the subjective opinion of the owners. regarding the appearance of clinical signs such as local pruritus, inflammation, recurrence of tumors, ulcers, etc.

Dogs of any breed or age can be affected, neutered or intact. The patients who were included in the study and benefited from treatment and investigations at the Faculty of Veterinary Medicine Cluj-Napoca, were divided into 3 main categories and another 8 subcategories: By sex and reproductive condition: neutered females (n = 6), intact females (n = 2), neutered males (n = 0), intact males (n = 4). By breed: pure breed dogs (n = 9), common breed dogs (n = 3). By age: Under 10 years: 6 patients with an average age of 6 years were included in this category. Over 10 years: 6 patients with an average age of 10.6 years were included in this category.

The physical examination of the skin formations was done systematically in order to identify the anatomical location, physical appearance, size, consistency, appearance of the adjacent tissues, skin-related lesions, adhesion to the underlying tissues and mobility.

All surgeries were performed under general anesthesia, based on well-established protocols, under the strict supervision of an anesthesiologist in the discipline. Patients were given specific anesthesia protocols and were assigned a degree of anesthesiological risk based on clinical examination and laboratory tests. Each patient had an intravenous catheter, which was mandatory. The steps of anesthesia were: pre-anesthesia in which the animal is sedated and immobilized, induction of anesthesia, endotracheal intubation, and maintenance of anesthesia was achieved by gas anesthesia (12).

The surgical technique was represented by the procedure of excision of the tumor formations with safety margins specific to each tumor type. This sometimes varies depending on the anatomical location of the tumor, the elasticity of the skin, the degree of infiltration, adhesion to underlying tissues, presence or absence of capsule, skin-related lesions, changes in regional lymph nodes. In 10 of the 12 patients studied, the technique was performed by surgical excision of the tumor mass with an underlying fascial plane and safety margins between 1 and 2.5 cm around the tumor. In the other two patients, in addition to this procedure, reconstruction techniques were used using skin flaps from the surrounding areas, followed by their transposition in place of the former tumor mass, which is possible due to the existence of blood vessels in the dermis. This was due to the fact that the anatomical location of the tumor mass was more difficult to approach and remedy, the skin was not in sufficient quantity, and mobility and elasticity were deficiency.

The first step was to mark the tumoral mass with a sterile marker and circle it at a distance of 1-2.5 cm around it. The second step was to cut the skin on the second line drawn with the marker, using the scalpel. Hemostasis was performed using the electrothermocautery. Using Metzembaum tissue scissors, the underlying tissue was torn, including a fascial plane, from close to close. The big vessels were ligated with 3-0 or 2-0 monofilament resorbable suture. After the ablation of the tumoral mass, in the 10 patients, the wound was closed. The subcutaneous connective tissue was torn all over the edge of the incision to promote the advancement of the skin, regarding the closure of the resulting defect. Backhaus staples were later used to close the edges of the wound and relax the skin the plans were sutured from the inside out. The first sutures aimed to close the edges of the wound and cancel the resulting space, thus preventing the formation of seroma. Walking sutures are performed for subcutaneous connective tissue with 3-0 and 2-0 monofilament resorbable. The skin suture was made according to the tension lines, in simple interrupted suture, at a distance of about 0.8 cm from each other with 2-0 monofilament non-absorbable. In the other cases, in addition to the surgical excision procedure, skin reconstruction techniques were performed to remedy the skin defects resulting from the extensive excision of the tumoral mass. In one case, the tumor mass was located on the face and the technique used for reconstruction was lip to lid flap. The resulting defect was remedied using a leather flap brought from the outer corner of the lip. Skin flap from the external angle of the lips can be used to cover facial facial defects in dogs and cats. This maneuver is based on the

existence of a collateral cutaneous branch, originating from the facial artery, *Angularis oris*, which is distributed at the level of the corner of the lips. In the other case, the tumor mass was located at the level of the lateral right hind limb. The technique used to remedy the resulting defect was flank fold flap. The skin flap from the flank was used to close the defect at the thigh, the side area, which was transposed at an angle of no more than 90 degrees. It is irrigated by the cranial and ventral branches of the deep iliac circumflex artery. Subsequently, the skin in both cases was sutured in separate interrupted pattern with 2-0 monofilament non-absorbable.

Results and discussions

During the study period, January 2019 - March 2021, the complete excision of skin tumors was performed in 12 dogs of several breeds: Amstaff (n = 1), Shar Pei (n = 1), Mops (Pug) (n = 1), Standard Schnauzer (n = 1), Siberian Husky (n = 1), Malamut of Alaska (n = 1), Dachshund (n = 1), Poodle (n = 1), Boxer (n = 1), Mixed breed dog (n = 3) (Table 1). Of the 12 patients included in the present study, the breed with the highest incidence of skin tumors was represented by mixed breed dogs (n = 3, 25%), comparable to other studies such as a retrospective study in Swiss in 2008-2013, where is stated that from a sample group of 11.740 dogs and the most frequently diagnosed with skin tumors were those of mixed breed (n = 2572, 21.91%) (6). The median age at the time of diagnosis of skin tumors and surgery was 6 years, in dogs under 10 years (between 3 and 10 years), and 10.6 years in those over 10 years (between 10 and 11 years). The study population consisted of 6 neutered females, 0 neutered males, 2 intact females and 4 intact males. A higher prevalence was observed in neutered females (n = 6, 50%), followed by the presence in intact males (n = 4, 33.3%), then in intact females (n = 2, 16.6%), and no castrated male. Similar results were published in the study mentioned above, showing that most skin tumors (n=3523; 30.01%) were encountered in female neutered dogs followed by intact males (n=3090; 26.32%) and female intact dogs (n= 2458; 20.94%). The smallest number of tumors was derived castrated males (n= 2224; 18.94%) (6).

The owners reported that the appearance of suspicious skin masses was observed no later than 18 months prior the clinical consultation. No patients in the study showed metastases or signs of general impairment. The skin tumors diagnosed in this study were: MCT (n = 5), Squamous cell carcinoma (n = 2), Infundibular cyst (n = 1), Hemangiopericytoma (n = 1), Peripheral nerve tumor (n = 1), sebaceous gland epithelium (n = 1), lipoma (n = 2). The most frequently diagnosed tumor was MCT (all grades) (n = 5, 41.6%), followed by squamous cell carcinoma (n = 2, 16.6%), infundibular cyst (n = 1, 8.3%), hemangiopericytoma (n = 1, 8.3%), peripheral nerve tumor (n = 1, 8.3%), sebaceous gland epithelium (n = 1, 3.3%), lipoma (n = 2, 16.6%), of which malignant (n = 9.75%) and benign (n = 3.25%). The high prevalence of MCT was not only observed in this study, there are

numerous retrospective studies where this tumor is in the top of diagnosed skin tumors, as in the study mentioned above where mast cell tumors (16.35% of all tumors), lipomas (12.47% of all tumors), and histiocytomas (12.10% of all tumors) were among the 4 most common tumor types, together with the group of hair follicle tumors (12.22% of all tumors) (6).

In 5 out of 12 patients, the diagnosis was based on this examination. The 5 patients were diagnosed with MCT, knowing that these tumors can be diagnosed in more than 96% of cases by cytological examination. At the cytological examination (Fig. 1), mast cells have a round, basophilic nucleus, including a multitude of purple cytoplasmic granules, many eosinophils may also be present (19). Some undifferentiated tumors may not contain granules, and the diagnosis may be made by histological examination (17). Only after histological examination can the degree of malignancy be established and a prognosis established. All patients benefited from postoperative histopathological examination of the tumor formation and safety margins, being also the method by which the definite diagnosis was established and on the basis of which a prognosis could be stated (Fig. 2).

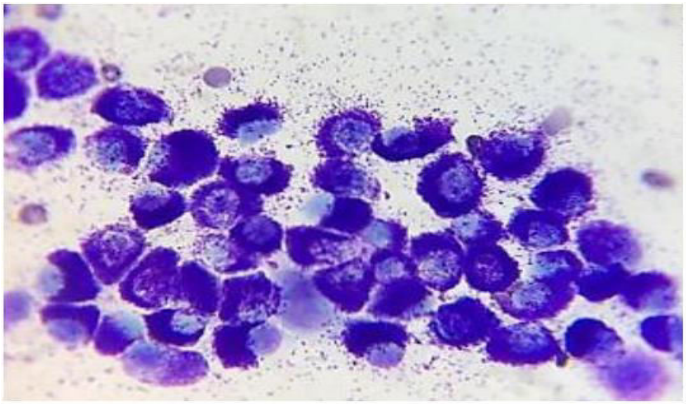


Fig. 1. Cytological examination of mast cell tumor - large, round cells with typical granules present in the purple cytoplasm with basophilic nucleus

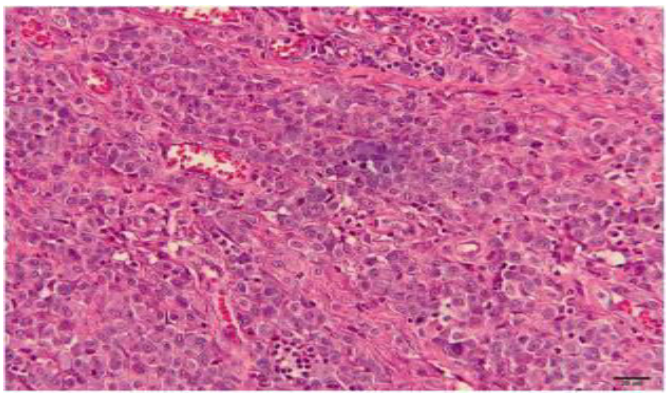


Fig. 2. Histological section in mast cell tumor

Formation intensely cellularized, located at the level of the dermis, poorly delimited, not encapsulated; it is composed of cells in the form of compact, round, oval, medium-sized cords with weakly distinct edges, moderate cytoplasm, and weakly acidophilic; average N / C ratio. The nuclei are large, round-oval arranged centrally or paracentral with fine-grained chromatin and 1-2 weakly visible nucleoli, basophilic granules being discreetly observed in the cytoplasm. Anisocytosis and anisokaryosis are severe, bizarre-looking nuclei (identified, sharp). Numerous multinucleated cells (> 10/10 HPF) are present. The mitoses are 0-1 / x40 microscopic field.

Our results are comparable to other studies in veterinary oncology on the incidence of skin tumors in dogs. A study conducted in Brazil on the incidence of skin tumors in dogs in El Salvador (2007-2016) diagnosed by histopathological examination, over a period of 10 years, on a population of 546 dogs, concluded that the most commonly diagnosed skin neoplasms are MCT and lipoma (11). On the other hand, another study conducted on a population of 1435 cases of skin tumors in Japan in the period 2008-2017, had the following results: Soft tissue sarcomas (18.40%), mast cell tumor (16.24%), lipoma (9.69%), hair follicle tumors (9.34%) and benign sebaceous tumors (8.50%) outperformed the others types of tumors. Tumors were commonly found on the head (13.87%), hind limbs (10.52%), forelimbs (8.01%), chest (5.78%) and neck (5.57%). The risk of developing skin tumors increased significantly in dogs aged 11 years and over ($P < 0.001$) (9). Another study evaluated the prognostic markers for hemangiopericytoma in 167 dogs. It assessed the postoperative outcome and the clinical parameters associated with the prognosis and the degree of recurrence depending on the surgical treatment performed and anatomical location. Following the results, he concluded that the initial aggressive surgical treatment is recommended to reduce the chance of tumor recurrence (10). The situation that we also faced in this study, so one of the dogs that was diagnosed with hemangiopericytoma 3 years ago and had surgical treatment at that time, suffered recurrence after resection with minimum safety margins.

The definite diagnosis of these was made within the Discipline of Pathological Anatomy, FMV Cluj Napoca, postoperatively by histopathological

examination. The cytological examination was able to guide the diagnosis for 5 of the 12 patients in the case of MCT, where it is known that these tumors can be diagnosed in more than 96% of cases by cytological examination (18). Regarding anatomical location, most were present in the limbs (n = 7, 58.33%), compared to other body sites. A similar situation was reported in a retrospective study in Korea from 2003-2006, where out of 748 cases of skin tumors diagnosed n = 66 were MCT of different degrees, and n = 27 representing 40.91% were present in the extremities (13).

Table 1

Summary of the study sample – NF: Neutered female, F: Female, M: Males

No	Breed	Age	Sex	Location	Lymph nodes reactions	DFI	MST	Diagnosis
1.	Mixed breed	7	NF	Parasternal	Unreacted	-	-	Mast cell tumor (low grade)
2.	Amstaff	3	M	Right hind limb	Unreacted	9	-	Mast cell tumor (high grade)
3.	Shar Pei	3	F	Left anterior limb	Unreacted	8	-	Mast cell tumor
4.	Mops (Pug)	7	M	Right hind limb	Unreacted	10	-	Mast cell tumor
5.	Standard Schnauzer	11	M	Right lateral scapular	Unreacted	-	-	Infundibular cyst
6.	Siberian Husky	11	M	Left anterior limb	Unreacted	2	-	Hemangiopericytoma
7.	Alaskan Malamute	10	NF	Head and left hind limb	Unreacted	12	-	Head-Peripheral nerve tumor Limb-Sebaceous gland epithelium
8.	Dachshund	8	NF	Sternal	Unreacted	-	-	Lipoma
9.	Mixed breed	10	NF	Sternal and abdominal	Unreacted	-	-	Lipoma
10.	Poodle	11	F	Head	Reacted	6	9	Squamous cell carcinoma
11.	Boxer	8	NF	Left hind limb	Unreacted	-	-	Mast cell tumor
m	Mixed breed	11	NF	Neck	Unreacted	5	-	Squamous cell carcinoma

The surgical technique performed for all patients was the excision of the skin tumoral masses with safety margins between 1 cm and 2.5 cm where the anatomical

location allowed this and an underlying fascial plane, followed by suturing the wound at interrupted pattern. Postoperatively, wound healing was achieved *per primam* in 10 patients, and in 2 of them a slight dehiscence of the wound was present and healed *per secundam*.

A re-examination was recommended at 3 months and 6 and 12 months, respectively, after surgery to assess skin function, local and general clinical signs. At the check-up, 7 of the dogs showed local clinical signs, but not general, and at the radiographic examination of the thoracic and abdominal cavity there were no visible densifications, attesting to the presence of metastases. At the end of the study, all the dogs were alive.

The disease-free interval was established in 7 dogs, which was 7.4 months (limits 5-12 months).

Conclusions

The study included a heterogeneous population of dogs, with skin tumors dominated by MCT of varying degrees.

At the end of the study, all the dogs were alive. The disease-free interval was established in 7 dogs, which was 7.4 months (limits 5-12 months).

Our results showed that the use of the tumor excision technique with safety margins specific to each tumor type and an underlying fascial plane is a viable treatment solution, even if a single treatment was used, without a complementary therapy (radiotherapy, chemotherapy). Surgical treatment of skin tumors restores normal skin function in the first 6 months postoperatively.

The results of this study complement the literature on the median survival time and the disease-free interval, being a study for the local population in Cluj-Napoca.

The limits of the study are represented by the small population observed and in fact it is a retrospective study, without standardization.

References

1. **Bostock, D.E.,** Neoplasms of the skin and subcutaneous in dogs and cats, British Veterinary Journal, 1986, 1, 142, 1-19.
2. **Brodey, R.S.,** Canine and feline neoplasia, Advances in Veterinary Science and Comparative Medicine, 1970, 14, 309-354.
3. **Dobson, J.M., Samuel, S., Milstein, H.,** Canine neoplasia in the UK: estimates of incidence rates from a population of insured dogs, Journal of Small Animal Practice, 2002, 43, 6, 240-246.
4. **Finnie, J.W., Bostock, D.E.,** Skin neoplasia in dogs, Australian Veterinary Journal, 1979, 55, 602-604.
5. **Gal, A.F., Cătoi, C., Tăbăran, F., Tăulescu, M.,** Anatomie patologică veterinară, Ed. AcademicPres, Cluj-Napoca, 2017.
6. **Graf, R., Pospischil, A., Guscetti, F., Meier, D., Welle, M., Dettwiler, M.,**

Cutaneous Tumors in Swiss Canine Cancer Registry 2008-2013, Veterinary Pathology, 2018, 55, 6, 809-820.
7. **Gross, T.L., Ihrke, P.J., Walder, E.J.,** Skin diseases of the dog and cat: Clinical and histopathologic diagnosis, 2nd edition, Blackwell Science Ltd: Oxford 2008.
8. **Kaldrymidou, H., Leontides, L., Koutinas, A.F.,** Prevalence, distribution and factors associated with the presence and the potential for malignancy of cutaneous neoplasms in 174 dogs admitted to a clinic in northern Greece, Journal of Veterinary Medicine Series A, 2002, 49, 87-91.
9. **Kok, M.K., Chambers, J.K., Tsuboi, M., Nishimura, R., Tsujimoto, H., Uchiela, K., Nakayama, H.,** Retrospective study of canine cutaneous tumors in Japan (2008-2017), Journal of Veterinary Medical Science, 2019, 81, 8, 1133-1143.
10. **Kravitz, A., Davis, G., Bastian, R.P., Fittipaldi, K.,** Outcome and Prognostic Indicators for Hemangiopericytomas in Dogs: 167 Cases (2009-2016), Journal of the American Animal Hospital Association, 2019, 55, 4, 194-200.
11. **Machado, G.A.C., Fontes, T.N., Larangeira, D.F., Estrela-Lima, A., Moreira, E.L.T., Ribeiro, L.S., Pinto, M.P.R., Peixoto, T.C.,** Incidence of skin tumors in dogs in Salvador, Bahia state, Brazil (2007-2016), Pesquisa Veterinaria Brasileira, 2018, 38, 11.
12. **Oana, L.I., Pestean, C.P., Ober, C.A.,** Ghid de anestezie si analgezie veterinara, Ed. Risoprint, Cluj-Napoca, 2011.
13. **Pakhrin, B., Kang, M.S., Bae, I.H., Park, M.S., Jee, H., You, M.H., Kim, J.H., Yoon, B.I., Choi, Y.K., Kim, D.Y.,** Retrospective study of canine cutaneous tumors in Korea, Journal of Veterinary Science, 2007, 8, 3, 229-236.
14. **Pakhrin, B., Kang, M.S., Bae, I.H.,** Retrospective study of canine cutaneous tumors in Korea, Journal of Veterinary Science, 2007, 8, 3, 229-236.
15. **Priester, W.A.,** Skin tumor in domestic animals. Data from 12 United States and Canadian colleges of veterinary medicine, Journal of the National Cancer Institut, 1973, 50, 457-466.
16. **Răpuntean, G., Răpuntean, S., Fiț, N.,** Imunologie veterinară, editura AcademicPres, Cluj-Napoca, 2008.
17. **Spencer, A., Johnston, K., Tobias, M.,** Veterinary surgery second edition, Vol. I., Elsevier, St. Louis, Missouri, 2018.
18. **Teifke, J.P., Lohr, C.V.,** Immunohistochemical detection of P53 overexpression in paraffin wax-embedded squamous cell carcinomas of cattle, horses, cats and dogs, Journal of Comparative Pathology, 1996, 114, 205-210.
19. **Teske, E.,** Clinical cytology of companion animals: part II, Cytology of subcutaneous swellings, skin tumours and skin lesions, European Journal of Companion Animal Practice, 2009, 19, 21-29.
20. **Vail, D.M., Thamm, D.H., Liptak, J.M.,** Small animal clinical oncology sixth edition, Elsevier, Saint Louis, 2020.

FELINE LOWER URINARY TRACT DISEASE: A RETROSPECTIVE STUDY

BĂRBOS A., BIRIȘ A., NEAGU D., MIRCEAN M., PUSTA D.

University of Agricultural Sciences and Veterinary Medicine,
Faculty of Veterinary Medicine, 400372,
3-5 Calea Manastur, Cluj-Napoca, Romania
E-mail: anca-dana.barbos@student.usamvcluj.ro

Summary

The Feline Lower Urinary Tract Disease (FLUTD) is a complex, often multifactorial pathology of clinical importance among domestic cats. It is characterized by inflammation with or without obstruction of the lower urinary tract with frequent clinical signs of dysuria, pollakiuria and hematuria. The present paper aims to evaluate epidemiological aspects of FLUTD in domestic cats by assessing 78 feline patients registered between January 2019 - November 2020 at the department of Internal Medicine, Faculty of Veterinary Medicine, Cluj-Napoca. The research intended to identify possible correlations between the incidence of FLUTD and predisposing factors represented by breed, sex, age, weight and season in which they presented for consultation. The results obtained in our study indicate that FLUTD predominantly affected European Shorthair cats (81%), males (90%), aged between 1 and 3 years (50%), weighing between 3 and 5 kg (50%). The maximum incidence was recorded during the cold season (33%).

Keywords: FLUTD, hematuria, stranguria, dysuria**.**

Feline lower urinary tract disease (FLUTD) is a complex, often multifactorial pathology of clinical importance among domestic cats. It is characterized by inflammation with or without obstruction of the lower urinary tract with frequent clinical signs of dysuria, pollakiuria and hematuria (9, 11). Another symptom usually noticed by the owners is represented by urinating in inappropriate places (periuria) due to increased pressure in the bladder caused by interstitial cystitis and urethritis (13, 18). These symptoms are not specific to a particular pathology and can be reported in urolithiasis, bacterial infections of the bladder or urethra, neoplasms or other bladder formations (2, 3). In approximately 2/3 of the cats that show these clinical signs, a definite etiological diagnosis cannot be established, so this syndrome is called feline idiopathic cystitis (CIF) (4, 19). Usually, the diagnose is set based on the clinical signs alone, but paraclinical investigations such as urine and serum biochemistry, urine sediment, urine cultures, ultrasound evaluations and radiography may be required for establishing the cause of FLUTD or to rule out other diseases.

Materials and methods

The epidemiological data included in the present study were provided by the written and electronic records of the Medical Pathology and Semiology Department

within the Faculty of Veterinary Medicine, Cluj-Napoca. We evaluated 78 feline patients registered between January 2019 - November 2020 at the department of Internal Medicine, Faculty of Veterinary Medicine, Cluj-Napoca that were presented for consultation with signs of FLUTD. The following variables were analyzed and recorded: the age and sex of the animals, the breed, the weight, and the season in which they were presented for consultation. The main breed represented in the study was European Shorthair, followed by British Short Hair, mainly males (70 out of 78) aged between 6 months and 13 years. The anamnestic data were obtained from the owners and in most cases consisted in general information about the patient, a history of FLUTD or the evolution of intercurrent diseases and the elapsed time between the apparition of symptoms and presentation for consultation. A complete general examination was performed by inspection, evaluation of vital functions by measuring respiratory and heart rate, thermometry, degree of hydration and evaluation by palpation of the fullness of the urinary bladder.

Results and discussions

Results are presented in Fig. 1. They showed that depending on the season in which the consultation was requested, it is noted that most of the illnesses were registered in the winter months (33%), respectively in January (11 cases) and in the spring months (26%), respectively in May (10 cases). Considerably fewer cases were identified in the summer (18%) and autumn (23%) months. Lekcharoensuk et al. (12) in a study from 2001, state that the highest incidence of FLUTD cases was in March and the lowest incidence was reported in August (12). Other studies mention that the incidence of FLUTD is higher during the months with lower temperatures and heavier rainfall because in these months cats do not leave the house as often and are prone to a sedentary lifestyle (11, 14, 20). All these aspects presented in the literature are confirmed by the present study.

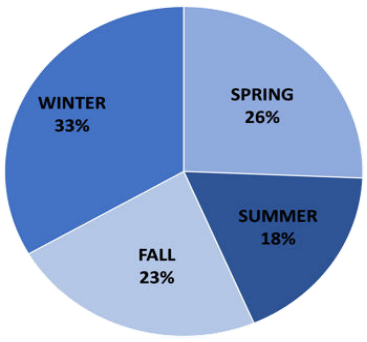


Fig. 1. Incidence of FLUTD depending on the season

Regarding the most affected breeds (Fig. 2), 63 of the 78 patients were identified as European shorthair (81%). Of the remaining 15 patients (19%), 4 were British Short Hair, 3 were Burmese, 2 were Persian, 2 were Siamese, 2 were Scottish Fold, 1 was Chartreux, and 1 was Russian Blue. This aspect is probably related to the fact that most of the patients presented for consultation were European shorthair cats. Some authors state that Persian, Himalayan and Manx cats are more prone, and Siamese cats are less likely to develop this condition (12), while others mention the increased incidence of the Persian cats (1).

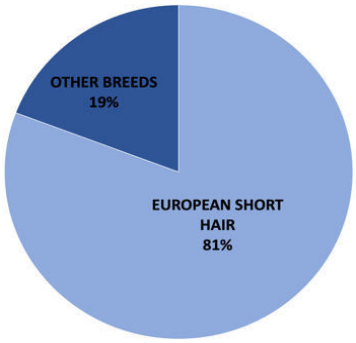


Fig. 2. Incidence of FLUTD depending on the breed

Regarding the patients' sex predisposition, our results showed that 90% were affected males and 10 % were females (Fig. 3). The data in the literature present different aspects regarding this topic. Some authors found the male predisposition (11, 20), while others support the hypothesis that females are more likely to develop FLUTD than males (1, 2, 17).

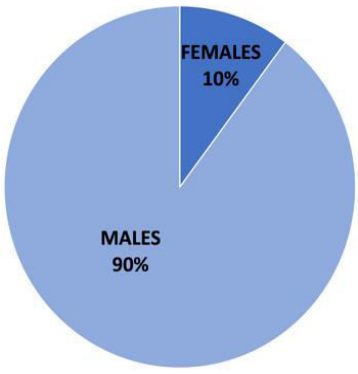


Fig. 3. Incidence of FLUTD depending on gender

Another important risk factor identified in this study is the patient's adult weight (Fig. 4). Of the 78 cats diagnosed with FLUTD, the highest share was represented by cats weighing between 3 and 5 kilograms (50%), and the lowest weight at those over 7 kilograms (5%). Given that most of the cats examined were European short haired, a weight of over 3 kilograms is considered above average, as they are considered overweight. Other studies mention in their studies that overweight cats are more likely to developing lower urinary tract disease (1, 2, 20). In another study it is also mentioned obesity as a predisposing risk factor, being associated with increased food intake, storage of higher fat and, consequently, increased excretion of minerals in the urine (15, 16).

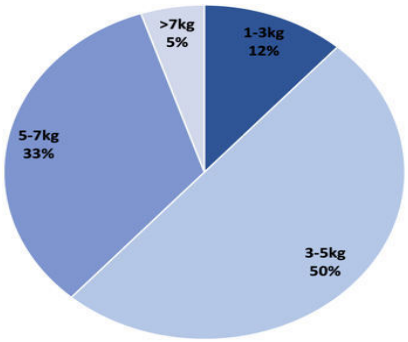


Fig. 4. Incidence of FLUTD depending on weight

Age is another important risk factor (Fig. 5). We noticed that most of the cases were between 1 and 3 years old (50%), followed by those between 4 and 6 years old (26%). Other authors mention a higher incidence among cats aged 4 to 7 years (6, 12, 15). In another study it is mentioned that cats over the age of 7 are most likely to develop FLUTD (10). Other studies report a higher incidence in patients older than 10 years (1, 5, 10).

Our results are not consistent with the data obtained by other researchers. We consider that the increased number of young patients affected by FLUTD (50%), is mainly produced because of their alimentation and overweight.

Of a total of 78 patients examined, 15 (19%) had episodes of FLUTD in the past, 5 (6%) had a gastrointestinal condition as a comorbidity, and 1 patient (1.3%) had nervous manifestations consisting of vocalizations, ataxia and seizures. In the present study, out of a total of 78 cats, 25 (32%) have been diagnosed with chronic kidney failure in the past. Other studies mention that 78% of cats showing signs of lower urinary tract disease have an associated comorbidity such as hyperthyroidism or chronic kidney failure (7, 8, 20).

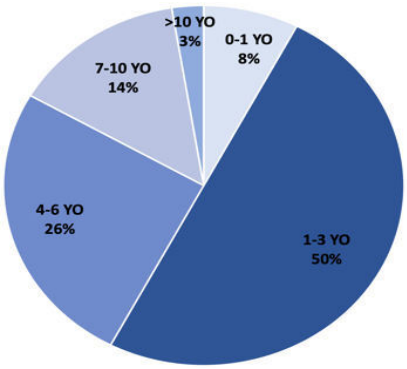


Fig. 5. Incidence of FLUTD depending on age

Conclusions

The present study was made on a total number of 78 patients diagnosed with FLUTD. The obtained results showed that the higher incidence of FLUTD was reported especially in the cold season, aspect that proves the implication of sedentarism in the pathophysiology of this disease. Regarding the breed predisposition, this pathology mainly affected European Shorthair cats. Most of the patients included in this study were young males, weighing between 3 and 5kg. 19% of the evaluated patients had a history of FLUTD, and in terms of comorbidities, 32% had a history of chronic kidney failure, 6% gastrointestinal disorders, and 1 patient presented with neurological signs.

We recommend a strict control of the diet, an active lifestyle, observing the behavior of water consumption and encouraging it.

References

1. **Bailiff, N.L., Westropp, J.L., Nelson, R.W., Sykes, J.E., Owens, S.D., Kass, P.H.,** Evaluation of urine specific gravity and urine sediment as risk factors for urinary tract infections in cats, Veterinary Clinical Pathology, 2008, 37, 3, 317-322.
2. **Bartges, J., Polzin, D.,** Nephrology and urology of small animals, John Wiley & Sons Wiley-Blackwell publishing, 2011.
3. **Buffington, C.A.T.,** External and internal influences on disease risk in cats. Journal of the American Veterinary Medical Association, 2002, 220, 7, 994-1002.
4. **Buffington, C.A., Chew, D.J., Kendall, M.S., Scrivani, P.V., Thompson, S.B., Blaisdell, J.L., Woodworth, B.E.,** Clinical evaluation of cats with

nonobstructive urinary tract diseases, Journal of the American Veterinary Medical Association, 1997, 210, 1.
5. **Byron, J.K.,** Urinary tract infection, Veterinary Clinics: Small Animal Practice, 2019, 49, 2, 211-221.
6. **Cannon, A.B., Westropp, J.L., Ruby, A.L., Kass, P.H.,** Evaluation of trends in urolith composition in cats: 5,230 cases (1985–2004), Journal of the American Veterinary Medical Association, 2007, 231, 4, 570-576.
7. **Davidson, A.P., Ling, G.V., Stevens, F., Franti, C.E., Johnson, D.L., Lang, S.S.,** Urinary tract infection in cats: a retrospective study 1977–1989, California Veterinarian, 1992, 46, 5, 32-34.
8. **Dorsch, R., von Vopelius-Feldt, C., Wolf, G., Mueller, R.S., Straubinger, R.K., Hartmann, K.,** Urinary tract infections in cats. Prevalence of comorbidities and bacterial species, and determination of antimicrobial susceptibility to commonly used antimicrobial agents, Tierarztliche Praxis, Ausgabe K, Kleintiere/heimtiere, 2016, 44, 4, 227-236.
9. **Gerber, B., Boretti, F.S., Kley, S., Laluha, P., Müller, C., Sieber, N., Unterer, S., Wenger, M., Flückiger, M., Glaus, T., Reusch, C.E.,** Evaluation of clinical signs and causes of lower urinary tract disease in European cats, Journal of Small Animal Practice, 2005, 46, 12, 571-577.
10. **Gregory, C.R., Vasseur, P.B.,** Long-term examination of cats with perineal urethrostomy, Veterinary Surgery, 1983, 12.4, 210-212.
11. **Jones, B.R., Sanson, R.L., Morris, R.S.,** Elucidating the risk factors of feline lower urinary tract disease, New Zealand Veterinary Journal, 1997, 45, 3, 100-108.
12. **Lekcharoensuk, C., Osborne, C.A., Lulich, J.P.,** Epidemiologic study of risk factors for lower urinary tract diseases in cat, Journal of the American Veterinary Medical Association, 2001, 218, 9, 1429-1435.
13. **Lew-Kojrys, S., Mikulska-Skupien, E., Snarska, A., Krystkiewicz, W., Pomianowski, A.,** Evaluation of clinical signs and causes of lower urinary tract disease in Polish cats, Veterinární Medicína, 2017, 62, 7, 386-393.
14. **Lopez, M., Hoppe, B.,** History, epidemiology and regional diversities of urolithiasis, Pediatric Nephrology, 2010, 25, 1, 49-59.
15. **Osborne, C.A., Lulich, J.P., Thumchai, R., Ulrich, L.K., Koehler, L.A., Bird, K.A., Bartges, J.W.,** 1996 Feline urolithiasis, Etiology and pathophysiology, Veterinary Clinic North American Small Animal Practice, 1996, 26, 2, 217-32.
16. **Palm, C.A., Westropp, J.L.,** Cats and calcium oxalate: strategies for managing lower and upper tract stone disease, Journal of Feline Medicine and Surgery, 2011, 13, 9, 651-660.
17. **Puchot, M.L., Cook, A.K., Pohlit, C.,** Subclinical bacteriuria in cats: prevalence, findings on contemporaneous urinalyses and clinical risk factors, Journal of Feline Medicine and Surgery, 2017, 19, 12, 1238-1244.
18. **Westropp, J.L., Bartges, J., Polzin, D.J.,** Feline idiopathic cystitis. Nephrology and Urology of Small Animals, Wiley-Blackwell Publishing, 2011.

19. **Westropp, J.L., Buffington, C.T.,** Feline idiopathic cystitis: current understanding of pathophysiology and management, Veterinary Clinics: Small Animal Practice, 2004, 34, 4, 1043-1055.
20. **White, J.D., Stevenson, M., Malik, R., Snow, D., Norris, J.M.,** Urinary tract infections in cats with chronic kidney disease, Journal of Feline Medicine and Surgery, 2013, 15, 6, 459-465.

ASSESSMENT OF BODY LANGUAGE (AGGRESSION) IN SHELTER DOGS

BOCHIȘ T., VOIA O., S., ȚIBRU I.

Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645,
Calea Aradului No. 119, Timisoara, Romania
E-mail: timea.bochis@usab-tm.ro

Summary

Dogs in shelters are subjected to a new environment, in which they are constantly exposed to stressors, interacting with people and foreign dogs. These factors lead to anxiety and aggressive defensive behavior. A number of 20 dogs (10 males and 10 females) were selected for this study, from a number of 200 dogs permanently housed in a shelter in the Municipality of Timişoara. This test aims to make an inventory and recognition of the specific behaviours of anxious, defensive dogs, which are prone to manifestation of aggression in this form, as a result of their accommodation in shelters. The behaviour of the dogs was videotaped, the images were subsequently processed and interpreted. The evaluation team consisted of two people, one evaluator and one person who registered. In order to determine if the evaluated dogs exhibit anxiety and defensive behavior, their response was assessed in three stages. At the end of the test, blood samples were taken from all individuals, from the cephalic vein in vacutainers without anticoagulant, to determine serum serotonin. The average serotonin values in the category of adoptable dogs was 251.25 ng / ml, they accepted the interaction with the evaluator, they were balanced dogs that did not show anxious behavior, nor other behavioural disorders. The category of dogs that showed fear, anxiety towards people through defensive posture, had an average serotonin values of 320 ng / ml, these individuals having a fearful attitude, behavioural therapy or socialization was recommended, depending on the individual needs of each dog. Extremely anxious dogs that exhibited aggressive behaviours were not considered suitable for adoption, the average serotonin values in this category was 450 ng / ml.

Keywords: dog, body language, serotonin, shelter.

Behavior evaluations play a critical role for shelter and rescue dogs, and are used to identify behavior tendencies in order to rehome an animal into an appropriate home.

Canine aggression is a normal behavior of the canine species, expressed differently, depending on the context in which it occurs (16, 18). This type of behavior can occur when the dog feels threatened, wants to impose his point of view, it can also be as an expression of a pathological condition or certain restrictions are imposed on the animal with which he does not agree (2, 15).

Human partners are an essential part of a dog's housing environment. Domestic dogs form attachment bonds with their owners (10), and the presence of the owner has been found to lower sympathetic arousal in threatening situations (7, 19). Human interaction programmes can reduce stress in shelter dogs. A central

function of the caretaker–dog relationship in prevention and treatment of behavioral problems of shelter dogs is plausible (3, 11, 16).

The human–animal relationship is defined as “the degree of relatedness or distance between the animal and the human, i.e., the mutual perception which develops and expresses itself in their mutual behaviour” (12, 20). A positive human–animal relationship is associated with pleasant emotions and rewarding events such as feeding or grooming, whereas a negative relationship is characterized by unpleasant emotions and aversive events such as rough handling (12).

The quality of the animal–human relationship can be assessed by measuring the animal’s behaviour towards a human. For example, fear of humans leads to increased avoidance and reduced approach behaviour in chicks, rabbits and heifers (8, 6, 9). The reaction of a dog towards humans might be influenced by quantity and quality of interactions with humans such as shared activities (2, 1) and use of training methods.

In the case of shelter dogs, positive human contact was shown to increase approach behaviour (5). Furthermore, a dog’s reaction to a potential adopter seems likely to influence the decision whether to adopt the dog. People who adopted a dog reported that when they first met the dog it approached them, licked them or jumped up (13); adopters preferred dogs who were quiet, alert, friendly and stayed at the kennel front (14).

Dogs in shelters are subjected to a new environment, in which they are constantly exposed to stressors, interacting with people and foreign dogs. These factors lead to aggressive defensive behavior (3).

This type of aggression will be observed in individuals who are anxious, insecure in certain circumstances, so as a defense, they will use aggression. If the warning signs are not understood by the shelter staff or others, the dog will bite without knowing the true cause (8).

The aggression out of fear occurs when the dog can no longer defend himself, but he cannot run away either. Then he will attack, no matter what the situation, even if it is a man (7).

Materials and methods

This test aims to make an inventory and recognition of the specific behaviors of anxious, defensive dogs, which are prone to manifestation of negative behaviours as a result of their accommodation in shelters.

A number of 20 dogs (10 males and 10 females) were selected for this study, 200 dogs permanently housed in a shelter in the Municipality of Timişoara. The selection criteria for dogs were: length of accommodation in the shelter (at least 6 months), age (1-7 years), sex, height, health (sick animals were excluded) and aggression. Information was collected about the history of the dogs' behavior: their reaction to staff, to strangers (visitors), the reaction to the approach of the person when they eat (accept / refuse), acceptance or not of physical contact with people.

If a dog exhibited negative behavior (growling, howling, biting) in one of these situations, it was considered that he could not be included in the experiment, as it is considered dangerous for the examiner.

In the selected group, out of a total of 20, there were stray dogs from the streets but also dogs abandoned by their owners for various reasons. The owners who brought the dogs to the shelter provided information on the behavioral history.

The examination method, respectively the behavior of the dogs was videotaped, the images were subsequently processed and interpreted. The evaluation team consisted of two people, one evaluator and one person who registered.

The dogs were housed in boxes, which housed a maximum of four dogs, in compliance with the space and welfare requirements according to the legislation in force.

The preliminary assessment took place in the accommodation boxes, each dog was observed for 10 minutes before being taken to the test room. The subjects were evaluated individually, then brought by the shelter staff to the evaluation room, which was 5x4 m in size.

At the end of the test, blood samples were taken from all individuals, from the cephalic vein in vacutainers without anticoagulant, to determine serum serotonin.

After being brought to the examination room, each dog was left for a period of 5 minutes to adjust to the place. During this time there was silence in the room, no discussion and no sudden movements.

In order to determine if the evaluated dogs exhibit aggressive defensive behavior, their body language was assessed:

- in the first stage, the evaluator sat down on a chair in a corner of the room. The dog, after entering the examination room, was allowed to get used to the new space, for a few minutes (3-5) depending on the temperament of each individual. The examiner during this time kept quiet, so as not to attract the dog's attention. The dog that approached the examiner in less than 30 seconds was marked with 3, the dog that approached after 30 seconds was marked with 2 and the dogs that did not approach at all, were marked with 1.
- in the second stage, the evaluator tried to establish eye contact with the dog, so the dogs that made eye contact in less than 5 seconds were marked with 3, those that allowed eye contact in 10 seconds received 2, and those who avoided visual contact received 1;
- in the third stage, an attempt was made to establish a physical contact between the evaluator and the dog. If the dog accepted a physical contact by stroking, it was marked with 3, if it accepted a prolonged physical contact, it was marked with 2, if it accepted a short physical contact, it was marked with 1. The final interpretation was based on the body language evaluated on the spot and based on the videorecording.

Results and discussions

In the first stage,

- the dogs marked with 3, were 8 (C1, C2, C6, C8, C10, C15, C17, C20), they approached the evaluator in less than 30 seconds, showed behaviors specific to the desire to socialize such as: they wagged tails when they saw the evaluator, raised their ears, looked lively, sniffed the evaluator. The mean serotonin was 289 ng/ml.
- 7 dogs were marked with 2, they approached the evaluator after 30 seconds (C3, C5 C9, C11, C13, C16, C19). The dogs expressed their desire to socialize, but only after they felt safe. The mean serotonin was 339 ng/ml.
- 5 dogs (C4, C7, C12, C14, C18) were marked with 1, they did not approach the examiner, after the accommodation period, they retreated to a corner of the room, behaviorally manifested by specific half moon eyes, c-shape body, tail between legs, adopting a fearful, anxious attitude. The mean serotonin values in these dogs were 427ng / ml (Fig.1, Table1).

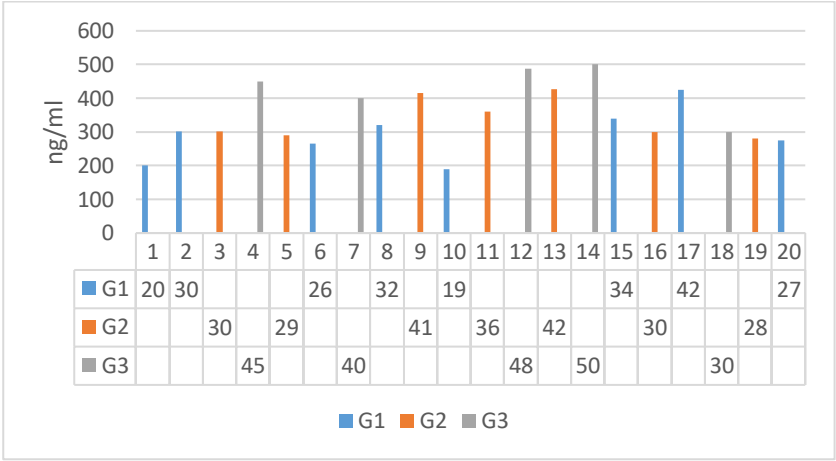


Fig.1. Results in the first stage

Analyzing the data obtained after the first stage, we say that 8 dogs responded favorably, by approaching the evaluator in less than 30 seconds, showing positive behaviors specific to the desire to socialize, 7 dogs approached the evaluator after 30 seconds but only after they felt safe and 5 dogs refused any contact with the evaluator, were part of the category of anxious dogs, who due to distrust in people prefer to withdraw (fig.1). For statistical analysis, Mann-Whitney U Test was used, in the first stage, the differences were significant between groups G1 and G3, $p \leq 0.05$ (Table 1).

Table 1

Significance of differences for stage 1

Grup	n	X ± s
G1	8	290 ± 76,25
G2	7	339 ± 61,32
G3	5	438 ± 81,29
G1 - G2		49 ns
G2 - G3		99 ns
G1 - G3		148 *

Notes: ns - insignificant p ≥ 0.05; * significant p ≤ 0.05

In the second stage,

•8 dogs (C1, C2, C5, C8, C10, C11, C15, C20), who responded favorably, received 3 points, they agreed to interact immediately, responding positive, making the eye contact immediately, these dogs enjoyed the comforts. Behaviorally, the dogs were relaxed, joyful and happy eyes, with energetic movements in their tails, some of them exhibiting playful behaviors. The mean serotonin levels in these dogs were 284 ng/ml.

•8 dogs (C3, C6, C9, C12, C13, C16, C18, C19) done the eye contact, but after 10 seconds, they were marked with 2, the average serotonin values were 347 ng/ml.

•4 dogs (C4, C7, C14, C17) avoided the eye contact, they were more confortable to stay in a corner of the room, they received 1. The mean serotonin was 443 ng/ml (Fig. 2, Table 2).

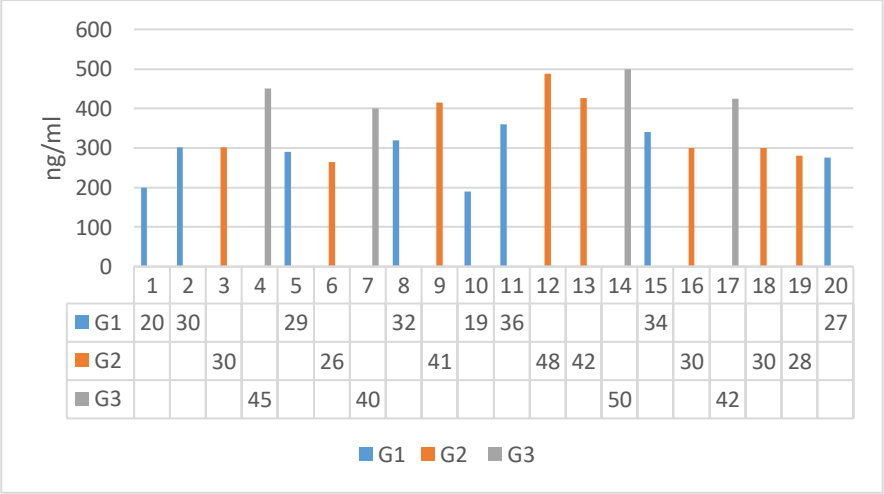


Fig. 2. Results in the second stage

Table 2

Significance of differences for stage 2

Grup	n	X ± s
G1	8	285 ± 61,53
G2	8	347 ± 83,15
G3	4	444 ± 42,70
G1 - G2		62 ns
G2 - G3		97 ns
G1 - G3		159 **

Note: ns - insignificant $p \geq 0.05$; ** significant $p \leq 0.01$

In the last stage,

•6 dogs (C1, C3, C5, C10, C15, C20) accepted the touch, without any negative behaviours or signs of aggression out of fear, for them it was a positive activity, so they received 3, and the average serotonin values were 266 ng/ml.

•8 dogs (C2, C4, C6, C9, C11, C12, C13, C18, C19,) who accepted the touch but showed signs of anxiety and were marked with 2, with an average serotonin value of 410 ng/ml.

•6 dogs (C7, C8, C14, C16, C17) accepted a short physical contact, were marked with 1 the mean serotonin values were 324.16 ng/ml (Fig. 3, Table 3).

Following the evaluation, at all stages only 4 dogs (C1, C10, C15, C20) were marked with 3, because they accepted the interaction with the evaluator, did not show anxious behavior, did not show muscle tension, the gaze was lively, playful, showed strong tail movements, were suitable for adoption, The mean serotonin values in this category of dogs was 251.25 ng/ml.

The dogs marked with 2 were 3 in number (C6, C9, C19), had a fearful behaviour, anxiety towards people through defensive posture, they moved away from the evaluator, turned their body in the opposite direction to which was approached, adopting a defensive physical posture: head lowered, ears on the back, muscles tense in the muzzle region, fearful gaze, center of gravity distributed on the hind limbs, tail retracted, curvature of the body line in the form of the letter C, piloerection in the tail region and The average serotonin levels in this category of dogs was 320 ng/ml.

Of the total number of dogs, only two (C7, C14) showed anxiety, were marked with a 1, showing a defensive posture expressed by obvious signs of discomfort in which they were. The mean serotonin values in this category were 450 ng/ml.

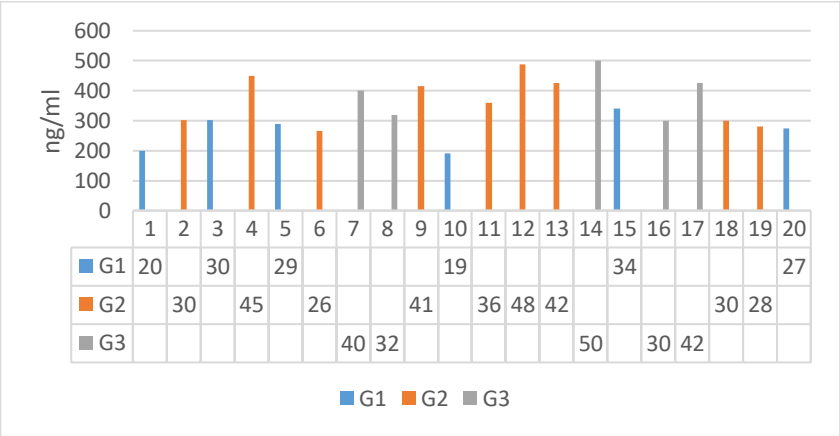


Fig. 3. Results in the third stage

Table 3

Significance of differences for stage3

Grup	n	X ± s
G1	6	266 ± 59,26
G2	9	365 ± 82,27
G3	5	389 ± 81,27
G1 - G2		99 ns
G2 - G3		24 ns
G1 - G3		123 *

Note: ns - insignificant p ≥ 0.05; * significant p ≤ 0.05

Conclusions

Dogs in shelters, due to the conditions in that environment, can respond in an unfavorable way, through defensive aggression. By knowing the body language, the recognition of the defensive behaviors of the dogs avoids the unpleasant situations when they become aggressive.

This type of aggression is due to anxiety, fear, but also previous experiences, and through behavioral therapy these behavioral disorders can be alleviated, but will be difficult to offer for adoption, because the degree of danger in the new environment.

The average serotonin values in the category of adoptable dogs was 251.25 ng/ml, they accepted the interaction with the evaluator, they were balanced dogs that did not show anxious behavior, nor other behavioral disorders.

The category of dogs that had showed fear, anxiety towards people through defensive posture, had an average serotonin values of 320 ng/ml, for these

individuals was recommended the behavioral therapy or socialization, depending on the individual needs of each dog.

Extremely anxious dogs that exhibited aggressive behaviors were not considered fit for adoption, the average serotonin values in this category was 450 ng/ml.

Comparing the values obtained in this study with the values obtained from shelter dogs in a previous study, which averaged 388.01 ng/ml, it can be stated that the lowest values of serotonin correspond to the individuals adapted to life from the shelter, they respond favorably to human interaction (251 ng/ml); average values of 320 ng/ml, were found in insecure individuals who are hesitant, and the highest values (450 ng/ml) were determined in individuals who showed obvious fear, anxiety, did not want any interaction.

References

1. **Arhant, C., Bubna-Littitz, H., Bartels, A., Futschik, A., Troxler, J.,** Behaviour of smaller and larger dogs: effects of training methods, inconsistency of owner behaviour and level of engagement in activities with the dog, Applied Animal Behaviour Science, 2010, 123, 131-142.
2. **Arhant, C., Troxler J.,** Approach behaviour of shelter dogs and its relationships with the attitudes of shelter staff to dogs, Applied Animal Behaviour Science, 2014, 160, 116-126.
3. **Bennett, P.C., Rohlf, V.I.,** Owner–companion dog interactions: relationships between demographic variables, potentially problematic behaviours, training engagement and shared activities, Applied Animal Behaviour Sciences, 2007, 102, 65-84.
4. **Bergamasco, L., Osella, M.C., Savarino, P., Larosa, G., Ozella, L., Manassero, M., Badino, P., Odore, R., Barbero, R., Re, G.,** Heart rate variability and saliva cortisol assessment in shelter dog: human–animal interaction effects, Applied Animal Behavior Sciences, 2010, 125, 56-68.
5. **Bollen, K., Horowitz, J.,** Behavioral evaluation and demographic information in the assessment of aggressiveness in shelter dogs, Applied Animal Behaviour Science, 2008, 112, 120-135.
6. **Christensen, E., Scarlett, J., Campagna, M., Houpt, K.,** Aggressive behavior in adopted dogs that passed a temperament test, Applied Animal Behaviour Science, 2007, 106, 85-95.
7. **Conley, M.J., Fisher, A.D., Hemsworth, P.H.,** Effects of human contact and toys on the fear responses to humans of shelter housed dogs, Applied Animal Behaviour Science, 2014, 156, 62-69.

8. **Csatadi, K., Kustos, K., Eiben, C., Bilko, A., Altbäcker, V.,** Even minimal human contact linked to nursing reduces fear responses toward humans in rabbits, Applied Animal Behaviour Science, 2005, 95, 123-128.
9. **De Meester, R.H., De Bacquer, D., Peremans, K., Vermeire, S., Planta, D.J., Coopman, F., Audenaert, K.,** A preliminary study on the use of the Socially Acceptable Behavior test as a test for shyness/ confidence in the temperament of dogs, Journal of Veterinary Behavior: Clinical Applications and Research, 2008, 3, 161-170.
10. **Gácsi, M., Topál, J., Miklósi, A., Dóka, A., Csányi, V.,** Attachment behavior of adult dogs (Canis familiaris) living at rescue centers: forming new bonds, Journal of Comparative Psychology, 2001, 115, 423-431.
11. **Hsu, Y., Sun, L.,** Factors associated with aggressive responses in pet dogs, Applied Animal Behaviour Science, 2010, 123, 108-123.
12. **Jones, R.B.,** Reduction of the domestic chick's fear of human beings by regular handling and related treatments, Animal Behaviour, 1993, 46, 991-998.
13. **Klausz, B., Kis, A., Persa, Esz., Miklósi, Á., Gácsi, M.,** A Quick Assessment Tool for Human Directed Aggression in Pet Dogs, Aggressive behavior Volume 9999, 2013, 1-11.
14. **Kottferovi, J., Marekovi, J., Jakuba, T.,** Aggressive behaviour of dogs and its ethological function, Folia veterinaria 2008, 73-74.
15. **Mazurek, M., McGee, M., Crowe, M.A., Prendiville, D.J., Boivin, X., Earley, B.,** Consistency and stability of behavioural fear responses of heifers to different fear-eliciting situations involving humans, Applied Animal Behaviour Science, 2011, 131, 21-28.
16. **Palmer, R., Custance, D.,** A counterbalanced version of Ainsworth's strange situation procedure reveals secure-base effects in dog–human relationships. Applied Animal Behaviour Science, 2008, 109, 306-319.
17. **Shiverdecker, M.D., Schiml, P.A., Hennessy, M.B.,** Human interaction moderates plasma cortisol and behavioral responses of dogs to shelter housing, Physiology and Behavior Journal, 2013, 109, 75-79.
18. **Waiblinger, S., Boivin, X., Pedersen, V., Tosi, M.V., Janczak, A.M., Visser, E.K., Jones, R.B.,** Assessing the human–animal relationship in farmed species: a critical review, Applied Animal Behaviour Science, 2006, 101, 185-242.
19. **Weiss, E., Miller, K., Mohan-Gibbons, H., Vela, C.,** Why did you choose this pet?: Adopters and pet selection preferences in five animal shelters in the United States, Animals 2, 2012, 144-159.
20. **Wells, D.L., Hepper, P.G.,** The influence of environmental change on the behaviour of sheltered dogs, Applied Animal Behaviour Science, 68, 2000, 151-162.

NEW PERSPECTIVES OF INTRAOCULAR ARTIFICIAL LENS IN DOGS - REVIEW ARTICLE

BONEA E., IGNA C.

Banat's University of Agricultural Sciences and Veterinary Medicine" King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645,
Calea Aradului No. 119, Timisoara, Romania
E-mail: drboneaelena@yahoo.ro

Summary

Cataract surgery is the only effective treatment of choice nowadays for restoring vision in dogs with cataract. Phacoemulsification technique with the implantation of an artificial intraocular lens (IOL) is currently the most desired treatment. IOL implantation improves the optics of the aphakic eye and reduces the formation of posterior capsular opacity (PCO) after surgery. There are 3 major classes of materials of IOLs.

Keywords: IOL, intraocular lens, dog, cataract, phacoemulsification.

When the dog's quality of life is being significantly affected by the loss of vision resulting from cataracts, the only effective and recommended treatment is surgery for extraction of diseased lens (3, 5, 10) and its replacement by an IOL (8, 9, 12, 14, 15). Cataract surgery has become very commonplace in humans to remove cataracts and restore eyesight in the affected eye(s) (9), with the first successful implant occurring in 1949 (1). Today over 6 million humans undergo this surgery every year. However, the first intraocular lens implants in dogs did not occur until 1991 (11).

Although there have been many discussions whether to implant IOLs in dogs or not (2), at present time most surgeons tend to implant (6, 8, 9, 14, 15).

Materials and methods

There are 3 classes of materials of IOLs: thermoplastics, synthetic elastomers and acrylate polymers (11). Polymethylmethacrylate (PMMA) is a thermoplastic polymer. It is light, durable, with a refractive index of 1.49. One-piece all-PMMA C-loop lens (Fig. 1-2) have the advantage of high flexibility - ideally suited for phacoemulsification, but the disadvantage of danger of postoperative decentration. There are one-piece IOLs c-loop haptic made with holes for fixing them in the capsular bag in order to prevent decentration. The circular 'all-in-the-bag' PMMA lens with flexible haptic, have the advantage of having a good compromise between flexibility and stability during compression optic and haptic remain at the same horizontal level - accurate and long-term centration. But as disadvantage, they are not suitable for phacoemulsification because of its wide diameter, around 8-9 mm.

The second class of IOLs includes the synthetic elastomers, the silicon lenses. Silicon is a biocompatible, flexible and elastic material, with a refractive index of 1.41-1.46, but they have lack of flexibility in comparison to PMMA (11).

Last class of IOLs materials include the acrylate polymers, the soft acrylic and collamers. Acrylate polymers are hydrogel polymers and the basis of all, is the acrylic hydrophilic monomer HEMA with a refraction index 1.43-1.48 (Fig. 3). Individual lenses differ in material composition and amount of water and one of the best tolerated lenses is monobloc (it is made out of 1 piece), with low content of water (Fig. 4).

Fig. 1. 1-piece PMMA C-loop haptic (20)

Fig. 2. One-piece all-PMMA IOL C-loop haptic design (original photo)

Fig. 3. Hydrophilic acrylic IOL (21)

Fig. 4. Monoblock hydrophilic acrylic foldable IOL (original photo)

Soft acrylic IOLs are hydrophobic lens with refractive index 1.47-1.55, these IOLs are biocompatible, foldable, with minimal occurrence of PCO.

The collamer IOLs are brand-new material with the combination of silicon and collagen (Fig. 5, 6).

Lenses can come with single-use preloaded injector for easy handling and insertion (Fig. 7), or can be used with a multiple-use injector, previously folded using IOL-holding forceps and inserted into the IOL cartridge (Fig. 8).

Fig. 5. One-piece PMMA, silicone and hydrophobic acrylate IOL (22)

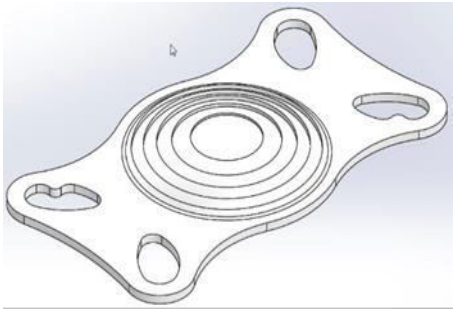


Fig. 6. Hydrophilic acrylate with hydrophobic surface dog IOL (18)

Fig. 7. Single-use IOL injector (original photo)

Fig. 8. Multiple-use Alcon Monarch II IOL injector (original photo)

Results and discussions

This collamers IOLs have high refractive index and excellent biocompatibility. These lenses are very thin, allowing implantation through incision even smaller than 3.2 mm.

Because silicon elastomers and acrylate/methacrylate polymers lenses are flexible, they are foldable lenses with the advantage that can be implanted through a small corneal incision (2.5-3.5 mm). Foldable lenses with this advantage are also the soft hydrophobic acrylic IOLs with PMMA mod C haptic (Fig. 9, Fig. 10).

No post operatory differences were observed between the one-piece IOL and haptic IOLs (17).

An-vision manufacturer developed an IOL made from hydrophilic biocompatible acrylate with a hydrophobic surface, 25% water content, incorporated UV-absorber, 360° continuous barrier around posterior side of optic to delay PCO, square edged haptic, and is the first canine IOL with extended depth of focus, with monofocal lens with D+41.0 that has an enhanced depth of focus of D +1.5 on one side and D -1.5 on the other side.

With this characteristics, most myopic and hyperopic dogs can be fully corrected, and emmetrope patient benefit from having better near sight (fig. 6) (18). Made from the same biocompatible, hydrophilic acrylate with a hydrophobic surface, the model MD6 from the same producer is the second the two-standard canine IOLs, also available in all sizes needed for dogs, but with haptic design in order to prevent vaulting in case of capsular shrinking. It has negative aberration in its optic to correct the positive aberration of the cornea like the natural lens (18).

Fig. 9. Soft hydrophobic acrylic IOL with blue PMMA mod C haptic (original photo)

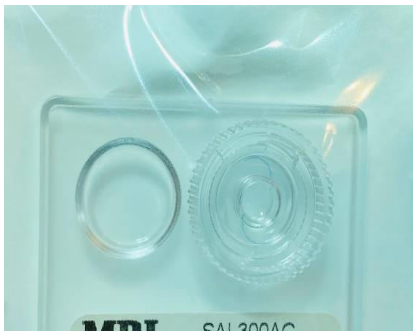


Fig. 10. Soft hydrophobic acrylic IOL with blue PMMA mod C haptic (original photo)

The surgical outcome and complications of phacoemulsification and the implantation of an acryl foldable intraocular lens with a squared edge in dogs with cataracts were analyzed and the complications after phacoemulsification were: posterior capsular opacity (PCO) around the IOL, ocular hypertension, focal posterior synechia, hyphema and corneal ulcer. The complications associated with the IOL were decentration of the optic and ventral haptic displacement. Most cases of PCO were found only around the margin of the IOL (13). When a 1-piece, tripod-shaped, acrylic intraocular lens was implanted in the posterior chamber after phacoemulsification to remove cataracts, complications were not encountered during implantation of the lens, but all dogs had mild ocular inflammation characterized predominantly by aqueous flare and mild corneal edema at day 1, which resolved completely by day 8 (6). Some complication like developed fibrin in

the anterior chamber, distorted pupils caused by adherence of vitreous humor or fibrin to the incision site, mild hyphema at day 1 or dislocated superior haptic can occur (6).

Conclusions

The important characteristics of IOLs are: density, refractive index, optical transmittance, dimensional stability, mechanical properties, biocompatibility, toxicity and chemical stability. An IOL must be simple to insert into the capsular bag, it must be extremely flexible and able to be securely fixed in position; it must therefore show a maximum of stability (7).

The collamer material is used for cataract as well as for refractive surgeries, due to its high refractive index and excellent biocompatibility. These lenses are very thin, thus allowing implantation through incision even smaller than 3.2 mm. Because silicon elastomers and acrylate/methacrylate polymers lenses are flexible, they are foldable lenses with the advantage that can be implanted through a small corneal incision (2.5-3.5 mm), also their excellent biocompatibility, we recommend them to be the first choice for phacoemulsification cataract, also minimizing astigmatism and post-surgery complications.

In order to achieve postoperative emmetropia, many researchers have documented the diopter (D) of dogs with an IOL to be approximately 40 D (7) and 41 D (3,4).

References

1. **Apple, D.J., Sims, J.,** Harold Ridley and the invention of the intraocular lens, Survey Ophthalmology, 1996, 40, 4, 279-92.
2. **Bigelbach, A.,** For and against lens implantation in dogs, Veterinary Quarterly 1994, 16, 1, 58S-59S.
3. **Davidson, M.G., Nasisse, M.P., Jamieson, V.E., English, R.V., Olivero, D.K.,** Phacoemulsification and intraocular lens implantation: a study of surgical results in 158 dogs, Progress in Veterinary and Comparative Ophthalmology, 1991, 1, 233-238.
4. **Davidson, M.G., Murphy, C.J., Nasisse, M.P., Hellkamp, A.S., Olivero, D.K., Brinkmann, M.C., Campbell, L.H.,** Refractive state of aphakic and pseudophakic eyes of dogs, American Journal Veterinary Research, 1993, 54, 174-177.
5. **Fischer, M.C., Meyer-Lindenberg, A.,** Cataracts in dogs--overview and guideline for decision making in treatment. TierarztIIche Praxis Ausgabe, K Kleintiere Heimtiere, 2014, 42, 6, 411-23.
6. **Gaidon, J.A., Lallement, P.E., Peiffer, R.L.,** Implantation of a foldable intraocular lens in dogs, Journal of the American Veterinary Medical Association, 2000, 216, 6, 875-7, 864.

7. **Gaidon, J.A., Rosolen, S.G., Steru, L., Cook, C.S., Peiffer, R.Jr.,** Use of biometry and keratometry for determining optimal power for intraocular lens implants in dogs, American Journal Veterinary Research, 1991, 52, 5, 781-3.
8. **Gilger, B.C., Whitley, R.D., McClauhlin, S.A., Wright, J.C., Boosinger, T.R.,** Scanning electron microscopy of intraocular lenses that had been implanted in dogs, American Journal Veterinary Research, 1993, 54, 1183-1187.
9. **Gilger, B.C., Whitley, R.D., McClauhlin, S.A., Wright, J.C., Boosinger, T.R.,** Clinical pathologic findings after experimental implantation of synthetic intraocular lenses in dogs, American Journal Veterinary Research, 1993, 54, 616-621.
10. **Gilger, B.C.,** Phacoemulsification technology and fundamentals, Veterinary Clinical North American, Small Animal Practice, 1997, 27, 1131-1141.
11. **Kecová, H., Neãas, A.,** Review Article. Phacoemulsification and Intraocular Lens Implantation: Recent. Trends in Cataract Surgery, Acta Veterinary Brno, 2004, 73, 85-92.
12. **Lim, C.C., Bakker, S.C., Waldner, C.L., Sandmeyer, L.S., Grahn, B.H.,** Cataracts in 44 dogs (77 eyes): A comparison of outcomes for no treatment, topical medical management, or phacoemulsification with intraocular lens implantation, Canadian Veterinary Journal, 2011, 52, 3, 283-8.
13. **Na-Young, Y., Shin-Ae, P., Man-Bok, J., Won-Tae, K., Se-Eun, K., Je-Min, C., Kang-Moon, S.,** Phacoemulsification and acryl foldable intraocular lens implantation in dogs: 32 cases, Journal of Veterinary Science, 2006, 7, 3, 281-285.
14. **Nasisse, M.P., Davidson, M.G., Jamieson, V.E., English, R.V., Olivero, D.K.,** Phacoemulsification and intraocular lens implantation in dogs: a study of technique in 158 dogs, Progress in Veterinary and Comparative Ophthalmology, 1990, 1, 225-232.
15. **Nasisse, M.P., Davidson, M.G., Jamieson, V.E., English, R.V., Olivero, D.K.,** Phacoemulsification and intraocular lens implantation: a study of technique in 182 dogs, Progress in Veterinary and Comparative Ophthalmology, 1991, 1, 225-232.
16. **Trivedi, R.H., Apple, D.J., Pandey, S.K., Werner, L., Izak, A.M., Vasavada, A.R., Ram, J.,** Sir Nicholas Harold Ridley, He changed the world, so that we might better see it, Indian Journal of Ophthalmology, 2003, 51, 3, 211-6.
17. **Zhong, X., Long, E., Chen, W., Xiang, W., Liu, Z., Chen, H., Chen, J., Lin, Z., Lin, H., Chen, W.,** Comparisons of the in-the-bag stabilities of single-piece and three-piece intraocular lenses for age-related cataract patients: a randomized controlled trial, BMC Ophthalmology, 2016, 16, 100.
18. ***https://www.an-vision.com/product/fox-iol-for-dogs
19. ***https://wagwalking.com/treatment/intraocular-lens-implants
20. ***https://m.made-in-china.com/product/One-Piece-PMMA-Intraocular-Lens-765252865.html

21. ***https://www.indiamart.com/proddetail/optima-veterinary-acrylic-foldable-intraocular-lenses-12211681273.html
22. ***https://www.indiamart.com/proddetail/360-degree-square-edge.html

ALTERNATIVE RECIPES FOR IMPROVING THE AVERAGE DAILY GAIN IN LAMB

CIREȘAN C.A.[1], CĂRPINIȘAN L.[1], LUCA I.[1], SÎRBU C.B.[1], IMRE M.[1], HOTEA, I.[1], MARIN A.M.[1], ONMAZ A.C.[2], DĂRĂBUȘ, GH.[1]

[1]Banat's University of Agricultural Sciences and Veterinary Medicine" King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645, Calea Aradului No. 119, Timisoara, Romania
[2]Erciyes University, Faculty of Veterinary Medicine, Department of Internal Medicine, Kayseri, 38100, Turkey
E-mail: alexandru.ciresan@usab-tm.ro

Summary

The weight gain of lambs is one of the most important indicators of production in sheep farming. The study presents comparatively different feeding recipes for infant lambs and their effectiveness in terms of average daily gain (ADG) performed on 3 groups of animals from the second week of life until the age of 6 weeks. In addition, the animals were parasitologicaly examinated to confirm the lack of parasitic elements that would induce erroneous results. Group 1 (control group) consumed only sheep's milk and voluminous feed (plain hay), group 2 consumed in addition to group 1 concentrated commercial feed from authorized producers, and lot 3 consumed in addition to group 1 concentrated feed produced in own regime having the main composition of corn and oats. The results show an advantage of ADG for the groups that also received concentrated feed and a better economic advantage in favor of the feed group with concentrated feed produced in own regime.

Keywords: average daily gain (ADG), lambs, corn, oats.

Globally, sheep are bred for various purposes: wool, milk, meat and fur production, the most important of which is meat production (6).

Sheep farming in Romania is an economic, family, mostly traditional activity with a strong social impact (17, 18). According to the centralized data at European level, Romania is on the podium when we talk about the number of sheep and the number of sheep exported both in Europe and in other continents (3). Among the breeds of sheep raised in Romania, the most important is *Tsurcana*, which represents approximately 70% of the total herd (13), the other breeds being represented by *Merinos of Palas*, *Merinos of Cluj*, *Tsigai*, *Ratca* etc.

Due to the growing demand for sheep meat, farmers are forced to find the most cost-effective solutions to increase the ADG (12, 19).

When we think about the economic effectiveness of raising animals, we must first consider their nutrition (16), so we must calculate very well the nutrition of sheep, the amount of feed they eat, the quality of feed and the degree of digestibility of both voluminous feed (20) and concentrated feed (7, 10).

As methods of improving ADG, farmers have resorted to either cross-breeding their domestic breeds with specialized breeds for meat (1, 9, 13, 14) or

improving feed recipes so that the maximum potential of their breed is reached (11, 15).

Materials and methods

The study was carried out on a farm in Timiș County, on three groups of animals, of 15 lambs of the *Tsucana* breed, starting from the age of 6-8 days (approximately one week) until the age of 6 weeks. The groups were formed as follows: 45 lambs (calved within 2-3 days), they were randomly put into groups as homogeneous as possible. Measurements started once the average age of the groups reached one week. The experiment was carried out in March-April 2022, when both adult sheep and their lambs were strictly sheltered.

Throughout the experimental period, the sheep received plain hay and water at their discretion, and in addition they received approximately 400 g of corn / animal. The lambs distributed in groups consumed the following:

- The control group (CG) consumed milk from their mothers, plain hay and water at will;
- Group 2 (G2) consumed in addition to CG 40 kg. of commercial granular concentrated feed designed by authorized producers;
- Group 3 (G3) consumed in addition to GC 40 kg of concentrated feed produced on the farm consisting of maize (49.5%), oats (49.5%), yeast (0.5%) and milk powder (0.5%).

During the experiment, the lambs were coprological tested weekly in order to exclude the posibility of parasitism that could have influenced the experiment. The method used was the Willis method (5).

The purchase price of the feed from the trade was 2 Lei / kg of feed, and the production cost of the own-produced feed was 1 Lei / kg of feed. The selling price of the lambs was 21 Lei / kg. All these prices were at the time of the study. They vary from month to month, depending on demand / supply.

Both purchased and self-produced concentrated feed were examined physico-chemically by the NIR method (2, 4) in order to observe their nutritional value.

Statistical calculations were performed using the statistical functions in Microsoft Excel, and statistical interpretation was performed using the Student Test (TTEST) (21).

Results and discussions

The result of the physico-chemical analysis of the concentrated feed, both commercially purchased and self-produced, is shown in Table 1.

Table 1

Physico-chemical composition of feed

Composition	**Feed purchased commercially**	**Feed produced on the farm**
Raw Protein **%**	18.20	10.77
Raw Fats **%**	3.40	5.23
Raw Cellulose **%**	9.18	5.33
Raw Ash **%** (total mineral)	4.80	2.20
Non-nitrogenous extractive substances (N .E.S.) **%**	52.19	63.07
Umiditat **%**	12.23	13.40
Dry matter %	87.77	86.6

The result of the weighing of the lambs for each week, on each group, can be found attached in Tables 2, 3 and 4.

Table 2

The evolution of the weight of the lambs from the CG

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Total kg. group	77	93.7	110.3	135.4	173	224.1
ADG (kg.)	-	0.1590	0.1580	0.2390	0.3580	0.4866
Mean	5.1333	6.2466	7.3533	9.0266	11.533	14.94
Standard Deviation	0.8796	1.1102	1.3994	2.0008	2.2843	2.5110
Standard Error	±0.2271	±0.2866	±0.3613	±0.5166	±0.5898	±0.6483

Table 3

The evolution of the weight of the lambs from G2

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Total kg. group	77.7	97.6	117	147.2	188.9	256.7
ADG (kg.)	-	0.1895	0.1847	0.2876	0.3971	0.6457
Mean	5.18	6.5066	7.8	9.8133	12.593	17.113
Standard Deviation	0.7291	0.6870	0.9935	1.3378	1.8167	2.3609
Standard Error	±0.1882	±0.1774	±0.2565	±0.3454	±0.4690	±0.6095

Table 4

The evolution of the weight of the lambs from G3

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Total kg. group	78.2	96	116.7	142.9	181.9	244.4
ADG (kg.)	-	0.1695	0.1971	0.2495	0.3714	0.5952
Mean	5.2133	6.4	7.78	9.5266	12.126	16.293
Standard Deviation	1.1685	1.5222	1.8229	2.1479	2.2776	2.6515
Standard Error	±0.3017	±0.3930	±0.4706	±0.5545	±0.5880	±0.6846

The differences between the evolution of the weight of the groups and the ADG can be seen in Fig. 1 and 2. There is a better ADG of G2 compared to the other groups, especially compared to the CG.

According to the Student test implemented in Microsoft Excel, the “p” value between G2 and G3 is over 0.05 (0.46 in the first week, decreasing to 0.18 in the last week) throughout the experiment, which proves that there are no significant differences between the two groups, although they received a different concentrated feed supplement.

* interpretation: - in order to have significant differences in comparison between the two groups, the value 'p' must be less than 0,05 (21).

No parasitic elements were identified following the coprological examination.

In the first week of the experiment there are no significant differences between the 3 groups due to the very low consumption of concentrated feed, these differences can be observed more and more accentuated starting after the second week of the experiment, until the end.

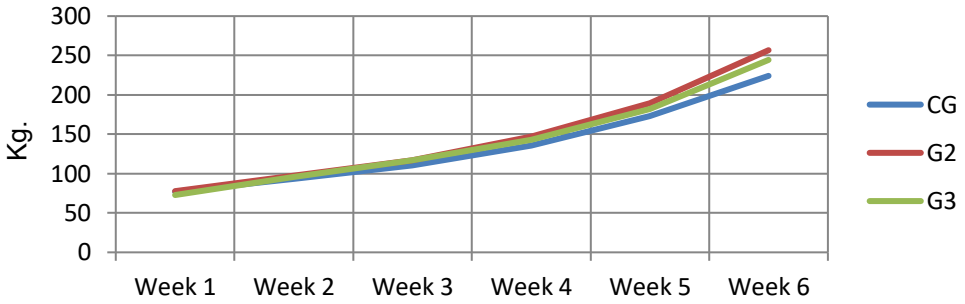


Fig. 1. Evolution of the weight of the groups over the period taken into account

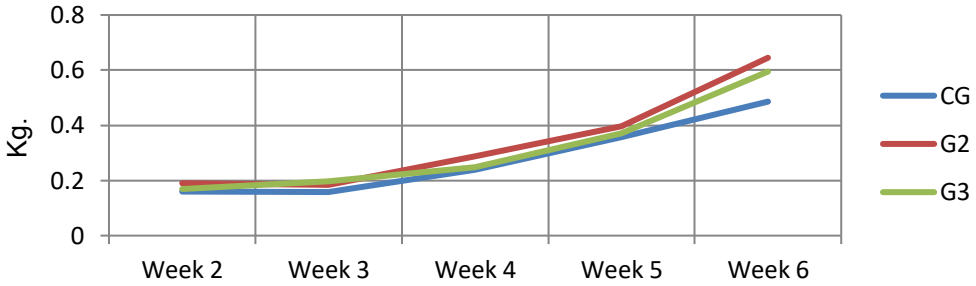


Fig. 2. The evolution of the ADG of the groups over the period taken into account

In the feed produced, powdered milk was added as a flavor enhancer to stimulate its consumption. *Yeast* was also introduced into this feed due to its inhibitory effect on *Clostridia* in the digestive tract of lambs (8).

The study showed an increase in weight of G2 compared to CG by 31.9 kg in total at the end, which meant that by investing 80 Lei for the purchase of feed, a profit of 669.9 Lei was produced (8.37 Lei for 1 Lei invested). And the difference between G3 compared to CG was 19.1 kg at the end which meant that by investing 40 Lei for feed production a profit of 401.1 Lei was achieved (10.02 Lei for 1 Leu invested).

Conclusions

Additional feeding of infant lambs with concentrated feed is beneficial for improving their ADG.

High quality concentrated feed (18% protein) produced the best ADG.

The concentrated feed we produced (10.77% protein) produced an economically satisfactory ADG, when we compare the acquisition costs with ADG of the two concentrated feeds at the time of the experiment.

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. **Burlacu, G.,** Methods and techniques for measuring the nutritional value of fodder, Ceres, Bucharest, 1991.
3. **Cazacu, C.,** Romanian livestock industry. Study on the effects of exports of bovine and ovine livestock vs. refrigerated/frozen meat export option, Original Media Bucharest, Romania, 2018.
4. **Dale, L.M.,** Determination of feed quality by destructive and non-destuctive methods, PhD Thesis, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 2011.
5. **Dărăbuș, Gh., Oprescu, I., Morariu, S., Mederle, N., Ilie, M.,** Practical guide in parasitic diseases, volume I, Ed. Agroprint, Timisoara, 2013.
6. **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.
7. **Hagos, T., Melaku, S.,** Feed intake, digestibility, body weight and carcass parameters of Affar rams fed tef (Eragrostis tef) straw supplemented with graded levels of concentrate mix, Tropical Animal Health and Production, 2009, 41, 599-606.
8. **Hstoum, R., Labrie, S., Fliss, I.,** Antimicrobial and probiotic properties of yeasts: from fundamental to novel applications, Frontiers in Microbiology, 2012, 3, 421, 1-12.
9. **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, 2010, 4, 60, 259-266.
10. **Jacques, J., Berthiaume, R. and Cinq-Mars, D.,** Growth performance and carcass characteristics of Dorset lambs fed different concentrates: forage ratios or fresh grass, Small Ruminant Research, 2011, 95, 2-3, 113-119.
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, Ciencia Animal Brasieira, 2014, 15, 4, 451-457.
12. **Pădeanu, I.,** Technical evaluation and genetical improvement of sheep productions, Ed. Mirton, Timisoara, 2003.
13. **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.
14. **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, 2011, 138, 1, 13-19.
15. **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.
16. **Stoica, I.,** Nutrition and animal nutrition, Ed. Coral Sanivet, Bucharest, 1997.
17. **Tafta, V.,** Rearing technologies for fattening lambs and goat-kids, Ed. Ceres, Bucharest, 2001.
18. **Tafta, V.,** Breeding sheep in small and medium sized farms, Ed. Ceres, Bucharest, 2003.
19. **Voia, S.O.,** Sheep and goats productions – Evaluation tehniques, Ed. Marineasa Timişoara, 2005.
20. **Wildeus, S., Turner, K.E. and Collins, J.R.,** Growth, intake, diet digestibility and nitrogen use in three hair sheep breeds fed alfalfa hay, Small Ruminant Research, 2007, 69, 1-3, 221-227.
21. ***https://www.researchgate.net/publication/277556608_Capitolul_5_Utilizarea_Excel_si_SPS S_in_statistica_sociala

BLUETONGUE EVOLUTION IN ISRAEL BETWEEN 2010 AND 2019 – AN EPIDEMIOLOGICAL APPROACH

COSTINAR L., HERMAN V., ABU M., BOCHIȘ T., PASCU C.

Banat's University of Agricultural Sciences and Veterinary Medicine" King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645, Calea Aradului No. 119, Timisoara, Romania
E-mail: corinapascu@usab-tm.ro

Summary

Currently, the presence of Bluetongue, an infectious disease with vector transmission (through *Culicoides*), is reported in many countries in Europe, Africa, the Middle East, Asia, as well as in Australia, and the USA. Given the importance of the disease, we considered it useful to address this research topic whose main purpose was to inventory cases of bluetongue in Israel over a period of 10 years, as well as the analysis of their distribution in order to be able to orient and focus, in the future, the prophylaxis measures. The cases were presented by region, to see where there is the greatest need for prevention and control measures. In addition, a case-by-case presentation of domestic and wild animals and a comparison of the total number of cases in Europe and Italy were provided. Were identified more serotypes in BT outbreaks in Israel. The most frequent serotypes were 2, 3, 4, 8, 9, and 16. Besides this, other serotypes identified were: 1, 12, 15, and 24. Association between serotypes 4, 8, and 12 in 2010 and 2 and 4 in 2012 was correlated with the highest number of outbreaks.The reemergence of high pathogenic serotypes of BTV and in the absence of a vaccine and control measures, the virus could spread to other countries, becoming a risk.
Keywords: Bluetongue, prevalence, Europe, serotypes, Israel.

Bluetongue is an infectious, non-contagious disease, also known as bluetongue. It is a viral disease (bluetongue virus VBT) with vector transmission (through *Culicoides*), and is found in domestic ruminants (sheep, goats, cattle) and wild (buffalo, deer). In most animals, the infection progresses unnoticed, but sometimes causes fatal cases in sheep, deer and wild ruminants. The disease is characterized by catarrhal or ulcer-necrotic inflammation in the nasal, oral and coronary mucosa and fever (13).

Currently, the presence of Bluetongue is reported in many countries in Europe, Africa and the Middle East, Asia, as well as in Australia and the USA. The disease causes serious economic losses due to various factors: mortality, fetal malformations, decreased productive capacity of animals, reduced cost of economic recovery of animals of susceptible species and products derived from them, costs of immunization of susceptible animals, trade restrictions (12, 22, 26).

Given the importance of the disease, we considered it useful to address this research topic whose main purpose was to inventory cases of bluetongue in Israel over a period of 10 years, as well as the analysis of their distribution in order to be able to orient and focus, in the future, the prophylaxis measures. The cases were

presented by region, to see where there is the greatest need for prevention and control measures. In addition, a case-by-case presentation of domestic and wild animals and a comparison of the total number of cases in Europe and Italy was provided. The official information that the Israeli authorities communicated to the international institutions in the field was used.

Materials and methods

To realize this paper, it was used data published on OIE (World Organization for Animal Health) website and data from WAHID (World Animal Health Information Database) (27). On this website were published information about Bluetongue outbreaks all over the world.

These data were completed with information published on Israeli Ministry of Agriculture (28).

In order to complete the overall picture of this disease, data were processed over a period of 10 years, so that in the event of registration errors, these errors are blurred and the analysis to capture as accurately as possible the real aspects of the disease in the territory. The dynamics of new cases (incidence) and total cases (prevalence) of this disease in Israel were monitored between 2010 and 2019. This study is ended in 2019 due to absence of any data in 2020 on OIE website and also on Ministry of Agriculture of Israel website.

Since the reporting of the evolution of Bluetongue in Israel was mainly done with the mention of the number of outbreaks and less of the number of affected animals, we considered it appropriate to refer to the number of outbreaks.

The Council Directive 82/894/EEC define outbreak “the holding or place situated in the territory of the country where animals are assembled and where one or more cases have been officially confirmed” (22).

The epidemiological study highlighted the steps that should be taken to limit the evolution over time of naturally occurring diseases, especially for Bluetongue.

Results and discussions

The obtained data from different sources and from literature were centralized and presented in tables and graphics as it follows.

The first outbreaks of Bluetongue were diagnosed in Israel in 1951, when six serotypes were detected: 2, 4, 6, 10, 15 and 16. Since then, Bluetongue has an endemic evolution, being declared new outbreaks year by year.

In 2008 Bluetongue was diagnosed with serotype 4 first in goats and, for the first time, cattle became diseased, with the same endemic serotypes 4 and 16.

Table 1

Total number of Bluetongue outbreaks in different species

Year	Cattle	Sheep	Cattle / Sheep / Goat (mixed herd)	Wild animals	Total
2010	48	12	16	2	**78**
2011	31	1	9	0	**41**
2012	13	11	80	0	**104**
2013	22	0	0	2	**24**
2014	0	6	7	0	**13**
2015	2	6	9	0	**17**
2016	3	7	19	0	**29**
2017	14	6	10	1	**31**
2018	11	5	6	0	**22**
2019	16	12	29	2	**59**
Total	**160**	**66**	**185**	**7**	**418**

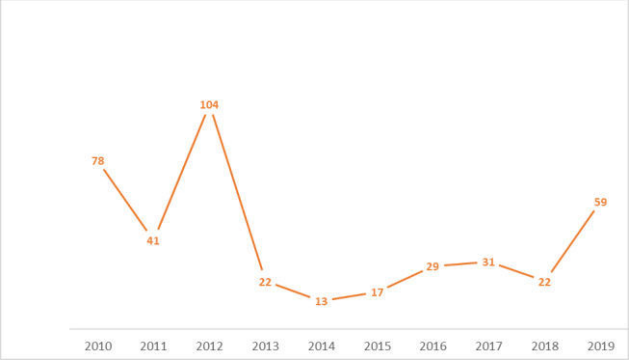


Fig. 1. Dynamics of total number of the Bluetongue outbreaks in Israel between 2010 and 2019

From data presented in table 1 and graph it could observe that the number of BTV outbreaks between 2010 and 2019 was variable in time, as it follows: was observed an initial decreased of the Bluetongue outbreaks number from 78 in 2010 at 41 in the next year. Beginning with 2012 the number of outbreaks increased sharply, being registered 104 outbreaks. In 2013 there is an equally sharp decrease in the number of outbreaks, being declared only 22. This decreasing trend was

maintained in the next year, with only 13 outbreaks. Between 2015 and 2017 the total number of outbreaks has a slight tendency to increase, with a highest number of 31 outbreaks. The outbreaks number decreased in 2018 at 22, but the number increased again in 2019, registering 59 new outbreaks.

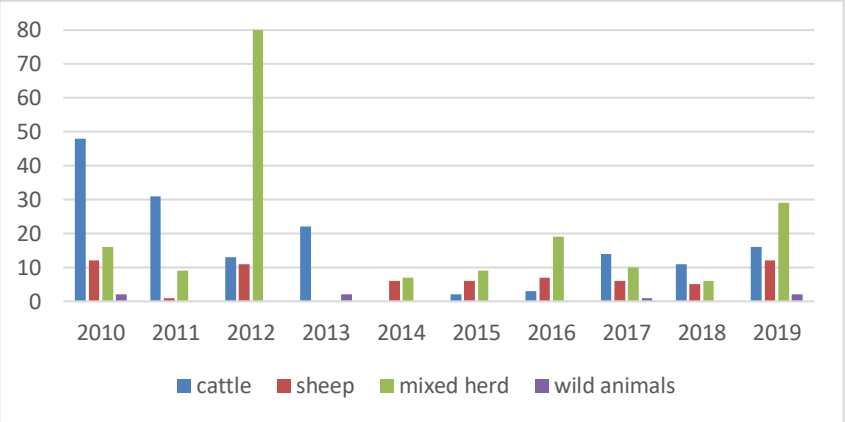


Fig. 2. The Bluetongue outbreaks on species

It observed that Bluetongue was diagnosed both in large and small ruminants. Cattle were more affected with 48 outbreaks in 2010, in 2011 with 31 outbreaks and in 2013, with 22 outbreaks. In 2014 no other outbreak was declared but starting with 2015 the number increase constantly in each year.

The number of outbreaks in sheep flocks variate less, the highest number of outbreaks was registered in 2010 and 2019 – 12 and in 2012 – 11 outbreaks. No outbreak was declared in 2013 and between 2014 and 2018 were declared less than 10 outbreaks.

In mixed herds the dynamic of disease was also variable, in 2012 registering the pick of the values, 80 outbreaks respectively. If in 2013 was not reported the disease on mixed farms, starting with 2014 it is observed an increase tendency until 2016, then the number decreased in 2017 and 2018, but it increased sharply in 2019 from 6 outbreaks in 2018 at 29 in 2019.

In fact, it can be seeing that 2019 is characterized by an increase in the number of outbreaks in all species sensitive at Bluetongue.

In wild species were declared only few Bluetongue outbreaks, in 2010, 2013, 2017 and 2019. The wild species affected were Eland and Mountain gazelle.

Analyzing data present in Table 2, which refers to BTV evolution in administrative division, it is observed that the highest number of outbreaks was registered in Hafazan, 215, where only in 2014 and 2015 were less than 10 registered outbreaks; and the lowest outbreaks number were registered in Haifa with 28 outbreaks, followed by Yerushalayim with 33 outbreaks.

Table 2

Number of BTV in administrative divisions

Admin.division/ year	Hafazan	Hadarom	Hamerzak	Haifa	Yerushalayim
2010	36	13	17	3	9
2011	20	13	6	2	0
2012	64	20	9	3	8
2013	13	3	6	0	0
2014	7	5	0	0	1
2015	7	3	4	1	2
2016	12	4	8	2	3
2017	16	9	0	3	3
2018	11	2	0	6	2
2019	29	9	8	8	5
Total	**215**	**215**	**215**	**215**	**215**

The outbreaks dynamic, as it is presented in Fig. 2 follows the same curves, with a decrease in 2011, with one exception – Hadarom, where the number remain the same as in previous year – 13 outbreaks. In Hamerzak and Haifa have been observed two consecutive years in which no new outbreak was registered. The presence of new outbreaks next year(s) demonstrate that the source of infection persisted in those regions and the control and prevention measures where not applied as the epidemiological situation require.

In 2019, increases were recorded in all administrative divisions.

More serotypes in BT outbreaks in Israel were identified (Table 3, Fig. 3.). The most frequently serotypes identified were 2, 3, 4, 8, 9 and 16. Besides this, other serotypes identified were: 1, 12, 15 and 24. Serotypes 2, 3, and 4 were identified in each year of study period, serotypes 8 and 9 identified in 2010, 2011, 2015, 2016 and 2018.

Association between serotypes 4, 8 and 12 in 2010 and between 2 and 4 in 2012 was correlated with the highest outbreaks number – 132 in 2010 and 103 in 2012, respectively.

In the case of identifying a single serotype, the highest number of cases were recorded in 2016, when there were 142 cases from 22 outbreaks, and the isolated viral serotype was 8 and in 2010 were 82 cases from 4 outbreaks.

Association of four serotypes, 4, 8, 12 and 24 respectively was correlated with the highest number of outbreaks, in 2010. The smaller number of outbreaks were produced by serotypes 4 and 16, in 2014.

Serotypes 2, 4, 6, 10, and 16 are considered endemic to Israel, all these serotypes could be identified in previous Bluetongue outbreaks. It is known form

literature (13) that serotypes 24, 16, and 12 are responsible for disease in cattle, and the high number of cases in cattle in 2010, 2011 and 2013 proved this.

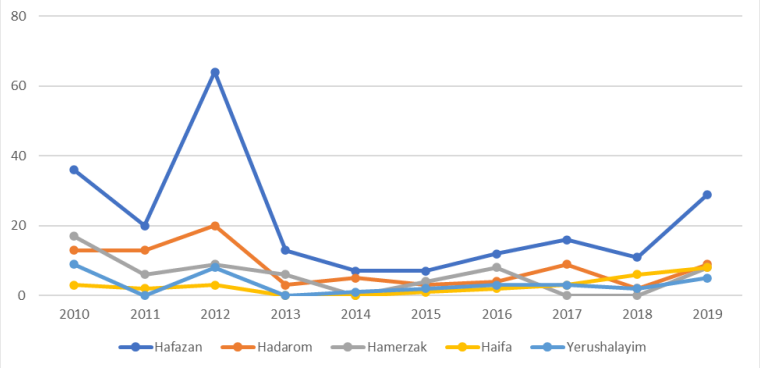


Fig. 3. Dynamics of Bluetongue outbreaks in administrative division of Israel

Studies realized by Brenner and col. (11) indicate that serotype 8 isolated in Israel is closely related with serotype 8 isolated in northern Europe having a common origin. The absence of a vaccine, could lead at reemergence of serotypes with high virulence and to severe losses.

Conclusions

The evolution of bluetongue in Israel has had an oscillating evolution during the 10 years, registering a peak of the number of outbreaks in 2012 with 104 outbreaks, followed by a decreasing number of outbreaks. This tendency was maintained until 2018 when was registered a new increase of the number of outbreaks, from 22 in 2018 at 59 in 2019. A positive correlation can be made between the large number on outbreaks and the number of identified serotypes.

Most cases in domestic animals have been reported in cattle.

The disease has also been reported in wild animals (eland and mountain gazelle), being registered 7 outbreaks in 2010, 2013, 2017 and 2019.

The region with the most cases was Hafazan, followed by Hadarom, and a positive correlation can be made between the large number of outbreaks and the migration corridors of the *Culicoides imicola*, the major African-Asian vector of Bluetongue virus.

The most frequently serotypes identified were 2, 3, 4, 8, 9 and 16. Besides this, other serotypes identified were: 1, 12, 15 and 24.

Reemergence of high pathogenic serotypes of BTV and in the absence of a vaccine and control measures, the virus could spread to other countries, becoming a risk.

References

1. **Al Ahmad, M.Z.A., Pellerin, J.L., Larrat, M., Chatagnon, G., Cécile, R., Sailleau, C., Zientara, S., Fieni, F.,** Can Bluetongue Virus (Btv) Be Transmitted Via Caprine Embryo Transfer?, Theriogenology, 2011, 76, 1, 126-132.
2. **Alexander, K.A., Maclachlan, N.J., Kat, P.W., House, C., O'brien, S.J., Lerche, N.W., Sawyer, M., Frank, L.G., Holekamp, K., Smale, L.,** Evidence of natural bluetongue virus infection among African carnivores, American Journal of Tropical Medicine and Hygiene, 1994, 51, 5, 568-576.
3. **Alphey, L.,** Genetic control of mosquitoes, Annual Review of Entomology, 2014**,** 59, 205-224.
4. **Al-Quraishy, S., Abdel-Ghaffar, F., Al-Rasheid, K.A., Mehlhorn, J., Mehlhorn, H.,** Effects of a neem seed extract (MiteStop®) on mallophages (featherlings) of chicken: *in vivo* and *in vitro* studies, Parasitology Research, 2012, 110, 617-622.
5. **Anderson, J., Hägglund, S., Bréard, E., Comtet, L., Lövgren, Bengtsson, K., Pringle, J., Zientara, S., Valarcher, J.F.,** Evaluation of the immunogenicity of an experimental subunit vaccine that allows differentiation between infected and vaccinated animals against bluetongue virus serotype 8 in cattle, Clinical and Vaccine Immunology, 2013, 20, 8, 1115-1122.
6. **Attoui, H., Maan, S., Anthony, S.J.,** Bluetongue virus, other orbiviruses and other reoviruses: Their relationships and taxonomy, 1st edition, Elsevier/Academic Press, London, UK, 2008.
7. **Audarya, S.D., Pandey, A.B., Nandi, S., Chauhan, R.S.,** Detection of bluetongue virus antibodies in goats of Delhi, India, Journal of Small Ruminants, 2015, 21, 1, 135-137.
8. **Backx, A., Heutink, C.G., Van Rooij, M.A., Van Rijn, P.A.,** Clinical signs of bluetongue virus serotype 8 infection in sheep and goats, Veterinary Record, 2007, 161, 17, 591-593.
9. **Batten, C.A., Harif, B., Henstock, M.R., Ghizlane, S., Edwards, L., Loutfi, C., Oura, C.A., El Harrak, M.,** Experimental infection of camels with bluetongue virus, Research in Veterinary Science, 2011, 90, 3, 533-535.
10. **Belbis, G., Zientara, S., Breard, E., Sailleau, C., Caignard, G., Vitour, D., Attoui, H.,** Bluetongue virus: From btv-1 to btv-27, Advances in Virus Research, 2017, 99, 161-197.
11. **Brenner, J., Batten, C., Yadin, H., Bumbarov, V., Friedgut, O., Rotenberg, D., Golender, N., Oura, C.A.,** Clinical syndromes associated with the circulation of multiple serotypes of bluetongue virus in dairy cattle in Israel, Veterinary Record, 2011,169, 389.
12. **Bumbarov, V., Golender, N., Jenckel, M., Wernike, K., Beer, M., Khinich, E., Zalesky, O., Erster, O.,** Characterization of bluetongue virus serotype 28, Transboundary and Emerging Diseases, 2020, 67, 1,171-182.

13. **Calvo-Pinilla, E., Nieto, J.M., Ortego**, **J.,** Experimental oral infection of bluetongue virus serotype 8 in type I interferon receptor-deficient mice, Journal of General Virology, 2010, 2821-2825.
14. **Carpenter, S., Wilson, A., Mellor, P.S.,** Culicoides and the emergence of bluetongue virus in northern Europe, Trends in Microbiology, 2009, 17, 172-178.
15. **Costinar, L., Herman, V., Iancu, I., Pascu, C.,** Phenotypic Characterizations and antimicrobials resistance of *Salmonella* strains isolated from pigs from fattening farms, Revista Română de Medicină Veterinară, 2021, 31, 2, 31-34.
16. **Golender, N., Bumbarov, V., Eldar, A., Lorusso, A., Kenigswald, G., Varsano, J.S., David, D., Schainin, S., Dagoni, I., Gur, I., Kaplan, A., Gorohov, A., Koren, O., Oron, E., Khinich, Y., Sclamovich, I., Meir, A., Savini, G.,** Bluetongue Serotype 3 in Israel 2013–2018: Clinical Manifestations of the Disease and Molecular Characterization of Israeli Strains, Frontiers in Veterinary Science, 2020, 7, 112-120.
17. **Golender, N., Panshin, A., Brenner, J., Rotenberg, D., Oura, C., Khinich, E., Bumbarov, V.,** Bluetongue virus serotype 24 (btv-24) in Israel: Phylogenetic characterization and clinical manifestation of the disease, Veterinaria Italiana, 2016, 52, 3-4, 333-341.
18. **Maclachlan, N.J.,** Bluetongue: History, global epidemiology, and pathogenesis, Preventive Veterinary Medicine, 2011, 102, 2, 107-111.
19. **Maclachlan, N.J., Drew, C.P., Darpel, K.E., Worwa, G.,** The pathology and pathogenesis of bluetongue, Journal of Comparative Pathology, 2009, 141, 1, 1-16.
20. **Maclachlan, N.J., Mayo, C.E., Daniels, P.W., Savini, G., Zientara, S., Gibbs, E.P.,** Bluetongue, Revue Scientifique et Technique, 2015, 34, 329-340.
21. **Mani, S., Singh, K.P., Jaynudin, H.K., Murali, D., Sobharani, V., Madhulina, M., At Faslu, R., Jyoti, M., Yashpal Singh, M., Vivek Kumar, G., Singh, R. K., Kuldeep, D.,** An updated review on bluetongue virus: epidemiology, pathobiology, and advances in diagnosis and control with special reference to India, Veterinary Quarterly, 2020, 40, 1, 258-321.
22. **Martin, A., Rajko-Nenow, P., Batten, C., Flannery, J.,** Simultaneous Detection of Bluetongue Virus Serotypes Using xMAP Technology, Microorganism, 2020, 8, 10, 1564.
23. **Simon-Loriere, E., Holmes, E.C.,** Why do RNA viruses recombine?, Nature Reviews Microbiology, 2011, 9, 617-626.
24. **Zientara, S., Sanchez-Vizcaino, J.M.,** Control of bluetongue in Europe, Veterinary Microbiology, 2013, 165, 33-37.
25. *** OIE (WAHIS) - https://wahis.oie.int/#/dashboards/qd-dashboard
26. *** Israeli Ministry of Agriculture https://www.moag.gov.il/vet/Yechidot/ Apidmiology/oie/PirsumimDochotOIE/2018/ Documents/doch_shnati_2017.pdf

PREVALENCE OF ZOONOTIC BACTERIA IN TERRESTRIAL AND AQUATIC TORTOISE FROM A ZOO PARK AND FROM OWNERS IN ROMANIA

COSTINAR L., HERMAN V., HULEA A., MERNEA I.O., PASCU C.

Banat's University of Agricultural Sciences and Veterinary Medicine" King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645,
Calea Aradului No. 119, Timisoara, Romania
E-mail: corinapascu@usab-tm.ro

Summary

The importance of reptiles in the transmission of microbial agents with zoonotic properties has increased greatly in recent times. It is well known that turtles can be carriers and eliminators of various species of *Salmonella* that can pose a risk to owners, carers and veterinarians. The most exposed to *Salmonella spp.* infection are children, the elderly or the immunocompromised. Contamination with *Salmonella spp*. of turtles can be done very easily, by overpopulated aquatics, with dirty water with feces or organic matter. The aim of this study was to determine the prevalence of *Salmonella* in water and land turtles from a zoo and private keepers, and to identify bacterial species isolated in association or not with *Salmonella*, especially those known to have zoonotic potential. Of the 41 turtles studied, 7.31% strains of *Salmonella spp*. were isolated, (3 strains) two of them being classified at the species level as belonging to the species enterica subsp. arizonae, and one could only be identified at the gender level. *Salmonella* strains were isolated from two specimens of semi-aquatic turtles (Trachemys scripta elegans) and from one specimen of land turtle (Testudo graeca). In addition to the isolated *Salmonella* strains, other bacterial genera were also identified, namely a *Morganella morgani* strain and a *Citrobacter braakii* strain. All species identified from turtles have zoonotic potential.

Keywords: Salmonella, prevalence, semi-aquatic turtles, zoonotic potential.

Purchasing turtles from stores without asking for or receiving information about their biology and pathology is most often followed by the negligent release of turtles into natural or semi-natural waters after they have grown or become "uncomfortable" for owners.

In Romania, specimens of *Trachemys* scripta *elegans* have been reported, a species considered exotic for our country, in natural or semi-natural habitats. This has led to the emergence of diseases transmitted by these exotic species to native species.

The importance of reptiles in the transmission of microbial agents with zoonotic properties has increased greatly in recent times (1, 9, 29). It is well known that turtles can be carriers and eliminators of various species of *Salmonella* that can pose a risk to owners, carers and veterinarians (3, 4, 5, 9, 12).

The most exposed are children, the elderly or the immunocompromised. Clinical evolution in humans can vary in severity, from gastrointestinal symptoms to severe infections and even death (22, 25, 27).

Despite the importance of *Salmonella* as the primary etiological agents in turtles and their risk as zoonotic agents, there is little information on the status of turtle carriers, and data in the literature vary from author to author (18, 20, 24, 26).

Contamination with *Salmonella spp.* of turtles can be done very easily, by overpopulated aquatics, with dirty water with feces or organic matter. Another permanent source of contamination is the introduction into livestock or trade of germ-carrying and eliminating animals, animals captured from the wild.

The aim of this study was to determine the prevalence of *Salmonella spp*. in water and land turtles in a zoo and private holders as well as to identify bacterial species isolated in association or not with salmon, especially those known to have zoonotic potential.

Materials and methods

For the research, a number of 41 turtles were studied, of which: 27 turtles were semi-aquatic, (belonging to the species *Emys orbicularis*, *Trachemis scripta elegans, Trachemis scripta scripta*) and 14 land turtles (belonging to the species *Testudo graeca*).

The turtles came from private breeders - 23 in number, from the Reșița Zoo - 13 in number and from the Timișoara Faculty of Veterinary Medicine - 5 in number.

The turtles at the Zoo were kept in a common pool, with access to the land surrounding the pool. The water was changed once a week. The food of turtles consists of liver and beef heart, fish and dry food for dogs. The diet also included lettuce leaves and, when possible, dandelion.

The semi-aquatic turtles that belonged to individuals were maintained in aquariums in variable numbers, from one to 3-4 turtles. Their diet consisted of chicken, fish and dry food for commercial turtles. In addition, they also received salad and fruit (apples).

Primary cultures were made from cloacal samples using sterile swabs. After collection, samples were kept at 4ºC and transported within 48 hours to the laboratory.

Before sampling, a rectal examination was performed with a sterile swab to induce urination. If collected directly with the swab for seeding, there was a risk that the swab would be contaminated with microorganisms from the urine, as reptiles almost always urinate at the time of cloacal collection.

For the isolation of species of the genus *Salmonella*, pre-enrichment and enrichment culture media were used, recommended by the standard SR EN ISO 6579 / A1, Amendment 1-Annex D: Detection of *Salmonella spp.* in faeces and environmental samples from the primary production stage (16, 29, 30).

The temperature at which the samples were incubated was different depending on the working stage and the culture medium used, respectively 37ºC and 42ºC for 24 hours and, if necessary, the incubation time was extended to 48 hours.

The identification of the strains was made based on morphological, cultural and biochemical characters on the media: Rappaport Vassiliadis broth, peptone water, Levine, MIU, SS, TSI and XLD, and the phenotypic characterization and taxonomic classification was performed using API 20E gallery (bioMerieux, France), and using the APIweb software.

Results and discussions

After examining the culture media used and the appearance of the colonies, the results shown below were obtained. In peptone water, after incubation for 24 hours at 37°C, all samples exhibited moderate to high turbidity. In Rappaport-Vassiliadis broth incubated at 42°C for 48 hours, *Salmonella* growth was different. After obtaining cultures in Rappaport-Vassiliadis broth, passages were made on special media.

Contrary to expectations, microorganisms belonging to other genera, such as *Morganella, Citrobacter* or *E. coli*, also grew in this broth, which was recognized as selective for *Salmonella*.

Thus, following the passage on the XLD environment, several types of colonies were obtained: pink colonies, with black center, the so-called “cat's eye”, specific to *Salmonella*, but also white-yellow colonies, of medium size. For a rapid differentiation of *Salmonella* strains from other enterobacteriaceae, especially against *E. coli*, passages on the Levine medium were subsequently performed.

The production of H2S and indole, as well as the fermentation capacity of sugars was highlighted on TSI and MIU media.

Because a precise identification of bacterial genera could not be made based on the cultural aspect, the API 20 E test was used for the biochemical differentiation of the isolated strains. The identification of *Morganella morgani* and *Citrobacter braakii* strains was made following the biochemical identification on API 20 E.

The prevalence of *Salmonella* in the turtles studied was 7.31%, respectively from the 41 turtles were isolated 3 strains of *Salmonella*, two of them being identified at the species level as *S. enterica subsp. arizonae*, and one was identified only at the gender level.

Salmonella strains were isolated from two specimens of Trachemys scripta elegans and one specimen of *Testudo graeca*.

The turtles from the Resita Zoo were negative for *Salmonella spp*.

Bibliographic data on the portability of *Salmonella spp.* in semi-aquatic turtles are quite different. Thus, some authors (7, 9) obtained a prevalence of *Salmonella* portage of 12%, a value close to that obtained in the research of this paper.

In contrast, other researchers obtained much higher *Salmonella* prevalence values of 38% (23) or 44% (16). In Japan in 2019, a rather high prevalence of *Salmonella* species was reported 57.3% in Trachemys s. elegans (12). In Spain, the

rate of isolation of *Salmonella* was very high from pet store samples 75.0% and moderate for private owners 29.0%. Serotyping revealed 18 different serotypes among two *Salmonella enterica subspecies*: *S. enterica subsp*. *enterica* and *S. enterica subsp. diarizonae.*

At the opposite pole, low prevalence values of 1.64% were also obtained (21). In our country, Kobolkuti obtained a prevalence of 13.63%, isolating 3 strains of *Samonella* from 22 semi-aquatic turtles (13). In contrast, they did not isolate any *Salmonella* strain from land turtles (16, 18).

These differences in the prevalence of *Salmonella* in turtles can be interpreted differently. In semi-aquatic turtles, the carrying of *Salmonella* could be influenced by the hygiene of the aquariums, the number of turtles in the aquariums and the diet. Uncontrolled and contaminated food (meat processing waste, fish, mice, earthworms) may contain or carry enteropathogenic *Salmonella*.

However, there is a fact that calls into question the previous assumptions, namely that the portage of *Salmonella* to land species is much higher than aquatic species. This could be due on the one hand to the aquatic environment in which the persistence of salmonella is lower, these being washed from the skin and cloaca of the turtles, and on the other hand to the mounting behavior of the land turtles, to which the females are mounted many males, salmonella can be transmitted through the cloacal route (7, 20, 24).

Along with *Salmonella arizonae* strains, well known as zoonotic, *Morganella morgani* and *Citrobacter braakii* strains increase the risk of human infections, also having zoonotic potential (3).

Conclusions

This study concluded that out of 41 turtles studied, 3 strains of *Salmonella* were isolated, of which two were classified at species level as belonging to *Salmonella enterica subsp. arizonae* and one could be identified only at genus level.

Salmonella strains were isolated from two semi-aquatic turtles (*Trachemys s. elegans*) and one terrestrial turtle (*Testudo graeca*).

In addition to the *Salmonella* strains isolated, other bacterial genera were identified, namely a *Morganella morgani* strain and a *Citrobacter braakii* strain.

All species identified in turtles have zoonotic potential.

The culture media used, in particular enrichment media (Rappaport-Vassiliadis broth, XLD agar) do not allow clear differentiation of Salmonella from other enterobacteriaceae, therefore final identification based on biochemical properties (API 20E test) or by molecular biological techniques is mandatory.

References

1. **Back, D.S., Shin, G.W., Wendt, M., Heoo, G.J.,** Prevalence of *Salmonella* spp. in pet turtles and their environment, Laboratory Animal Research, 2016, 32, 166-170.
2. **Basler, C., Bottichio, L., Higa, J., Prado, B., Wong, M., Bosch, S.,** Multistate outbreak of human *Salmonella* Poona infections associated with pet turtle exposure-United States, 2014, Morbidity and Mortality Weekly Report, 2015, 64, 804.
3. **Braun, S., Spalloni, W., Ferreccio, F., Postigo, J., Fernández, A., Porte L., Saldivia, A., Wigant, W., Triantafilo, V.,** *Salmonella* spp. gastroenteritis associated to pet turtles in three infants, Revista Chilena de Infectologia, 2015, 32, 334-338.
4. **Costinar, L., Herman, V., Iancu, I., Pascu, C.,** Phenotypic Characterizations and antimicrobials resistance of *Salmonella* strains isolated from pigs from fattening farms, Revista Română de Medicină Veterinară, 2021, 31, 2, 31-34.
5. **De Jong, B., Andersson, Y., Ekdahl, K.,** Effect of regulation and education on reptile-associated salmonellosis, Emerging Infectious Diseases, 2005, 11, 398-403.
6. **Franco, A., Hendriksen, R.S., Lorenzetti, S., Onorati, R., Gentile, G.,** Characterization of *Salmonella* occurring at high prevalence in a population of the land iguana Conolophus subcristatus in Galápagos Islands, Ecuador, 2011, PLoS One, 6, e23147.
7. **Geue, L., Löschner, U.,** *Salmonella* enterica in reptiles of German and Austrian origin, Veterinary Microbiology, 2002, 84, 79-91.
8. **Harris, J.R., Neil, K.P., Behravesh, C.B., Sotir, M.J., Angulo, F.J.,** Recent Multistate Outbreaks of Human *Salmonella* Infections Acquired from Turtles: A Continuing Public Health Challenge, Clinical Infectious Diseases, 2010, 50, 554-559.
9. **Herman, V., Pascu, C., Grema Fini, C., Marcu, A., Surpat, A., Tătar, D.,** The seroprevalence of *Salmonella spp*. in association with Swine Influenza Virus H1N1 in pig farms located in Western Romania, International Scientific Congress - Soil and food, resources for a healthy life, Iași, 2015, 33.
10. **Hoelzer, K., Moreno Switt, A.I., Wiedmann, M.,** Animal contact as a source of human non-typhoidal salmonellosis, Veterinary Research, 2011, 42-34.
11. **Kobolkuti, L., Gábor, Á., Spînu, M.,** Effects of malnutrition and improper captive maintenance on European pond turtle (Emys orbicularis): a case report, Journal of Animal and Plant Sciences, 2016, 874-879.
12. **Kuroki, T., Ito, K., Ishihara, T., Furukawa, I., Kaneko, A., Suzuki, Y., Seto, J., Kamiyama, T.,** Turtle-Associated *Salmonella* Infections in Kanagawa, Japan, Japanese Journal Infectious Diseases, 2015, 68, 333-337.

13. **Marin, C., Ingresa-Capaccioni, S., González-Bodi, S., Marco-Jiménez, F., Vega, S.,** Free-living turtles are a reservoir for *Salmonella* but not for *Campylobacter*, PLoS One, 2013, 8:e, 72350.
14. **Marin, C., Vega, S., Marco-Jiménez, F.,** Tiny Turtles Purchased at Pet Stores are a Potential High Risk for *Salmonella* Human Infection in the Valencian Region, Eastern Spain, Vector Borne*,* Zoonotic Diseases, 2016, 16, 455-460.
15. **Nakadai, A., Kuroki, T., Kato, Y., Suzuki, R., Yamai, S.,** Prevalence of *Salmonella* spp. in pet reptiles in Japan, Journal of Veterinary Medical Science, 2005, 67, 97-101.
16. **Pascu, C., Costinar, L., Mernea, I., Tătar, D., Herman, V.,** Prevalence of *Lawsonia intracellularis* infections in pig herds from the Western Romania, International Conference „Agriculture for Life, Life for Agriculture" București, 2015, 104.
17. **Readel, A.M., Phillips, C.A., Goldberg, T.L.,** Absence of cloacal shedding of *Salmonella* in wild red-eared sliders (Trachemys scripta elegans), Herpetological Review, 2008, 39, 427-430.
18. **Readel, A.M., Phillips, C.A., Goldberg, T.L.,** Prevalence of *Salmonella* in Intestinal Mucosal Samples from Free-ranging Red-Eared Sliders (*Trachemys scripta elegans*) in Illinois, Herpetological Conservation and Biology, 2010, 5, 207-213.
19. **Saelinger, C.A., Lewbart, G.A., Christian, L.S., Lemons, C.L.,** Prevalence of *Salmonella* spp in cloacal, fecal, and gastrointestinal mucosal samples from wild North American turtles, Journal of the American Veterinary Medical Association, 2006, 229, 266-268.
20. **Sánchez-Jiménez, M.M., Rincón-Ruiz, P.A., Duque, S., Giraldo, M.A., Ramírez-Monroy, D.M.,** *Salmonella enterica* in semi-aquatic turtles in Colombia, Journal of Infection in Developing Countries, 2011, 5, 361-364.
21. **Santoro, M., Hernandez, G., Caballero, M.,** Aerobic bacterial flora of nesting green turtles (Chelonia mydas) from Tortuguero National Park, Costa Rica, Journal of Zoo and Wildlife Medicine, 2006, 37, 549-552.
22. **Siebeling, R.J., Neal, P.M., Granberry, W.D.,** Evaluation of methods for the isolation of *Salmonella* and Arizona organisms from pet turtles treated with antimicrobial agents, Applied Microbiology, 1975, 29, 2, 240-245.
23. **Smith, K.F., Yabsely, M.J., Sanchez, S., Casey, C.L., Behrens, M.D., Hernandez, S.M.,** *Salmonella* isolates from wild-caught Tokay geckos (Gekko gecko) imported to the U.S. from Indonesia, Vector Borne Zoonotic Diseases, 2012, 12, 575-582.
24. **Sodagari, H.R., Habib, I., Shahabi, M.P., Dybing, N.A., Wang, P., Bruce, M.,** A Review of the Public Health Challenges of *Salmonella* and Turtles, Veterinary Science, 2020, 7, 2, 56-60.
25. **Torfoss, D., Abrahamsen, T.G.,** *Salmonella* infection from turtles, Tidsskrift for the norske Laegeforening, 2000, 120, 3670-3671.

26. **Van Meervenne, E., Botteldoorn, N., Lokietek, S., Vatlet, M., Cupa, A., Naranjo, M., Dierick, K., Bertrand, S.,** Turtle-associated *Salmonella* septicaemia and meningitis in a 2-month-old baby, Journal of Medical Microbiology, 2009, 58, 1379-1381.
27. **Woodward, D.L., Khakhria, R., Johnson, W.M.,** Human salmonellosis associated with exotic pets, Journal Clinical Microbiology, 1997, 35, 11, 2786-2790.
28. ***CDC Centers for Disease Control and Prevention. Turtle-associated Salmonellosis in humans – United States, 2006-2007.
29. ***CDC Centers for Disease Control and Prevention. Multistate outbreak of human *Salmonella typhimurium* infections associated with pet turtle exposure – United States, 2008, MMWR Morbidity and Mortality Weekly Report, 2010, 59, 191-196.
30. ***EFSA European Food Safety Authority. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010, EFSA Journal, 2012, 10, 2597.

EPILEPTIC DISCHARGES IDENTIFIED IN A CANINE PATIENT WITH QUADRIGEMINAL CYST BUT UNRELATED TO LOCALIZATION OR TO THE PATIENT'S EVOLUTION UNDER CONSERVATIVE TREATMENT

DEMÉNY H.[1], FLOREA B.[2], LOGIN C.C.[3], DOROBANȚU F.R.[4], OGNEAN L.[1]

[1]USAMV Cluj-Napoca, Facultatea de Medicina Veterinara,– 400372, Calea Manastur 3-5, Cluj-Napoca, Romania
[2]Epilepsy and Electroencephalography Monitoring Center, Romania
[3]Department of Physiology, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj -Napoca, Romania
[4]Department of Pathophysiology, Faculty of Medicine and Pharmacy, University of Oradea, Romania
E-mail: demeny.helga@yahoo.com

Summary

Congenital intracranial arachnoid cysts have been described in humans and dogs. Quadrigeminal cysts represent the most common type of intracranial arachnoid cysts in dogs, primarily affecting males and small brachycephalic breeds. Frequently, quadrigeminal cysts represent an incidental finding; their clinical significance in dogs and the best therapeutic strategies for these canine patients remain controversial. We describe here electroencephalogram (EEG) changes observed in a dog with epileptic seizures, in which a QC was identified by magnetic resonance imaging (MRI). We attempted to establish a correlation between the localization of electrical epileptic foci and that of the QC, and to evaluate the value of EEG monitoring in assessing clinical evolution under conservative treatment.

Keywords: EEG, epileptic discharges, quadrigeminal cyst.

Congenital intracranial arachnoid cysts (IACs) represent developmental brain lesions formed through a focal splitting or duplication of the leptomeninges that results in a diverticulum filled with cerebrospinal fluid (CSF) (4, 14, 17).

Cases of IACs have been described in humans and dogs (1, 3, 4, 5, 10, 12-17). While in humans, IACs can be localized in several areas (4,17), in dogs IACs occur almost exclusively in the caudal fossa, associated with the quadrigeminal cistern; for this reason, IACs are called quadrigeminal cysts (QCs) in this species (4, 12, 14). The prevalence of QCs in the general canine population is low; Matiasek et al. (9) foundthat only <0.7% of all patients with suspected intracranial lesions (4100 dogs) wereidentified as having QCs.

Based on the cases reported in the literature, QCs appear to occur with predilection in small-breed, brachycephalic, male dogs (10, 12, 14, 15). In many cases, a QC represents an incidental finding, therefore the clinical significance and the best therapeutic strategies for the patients with such pathology still remain controversial (4, 5, 12). To our knowledge, this is the first publication to assess the

appearance of the electroencephalogram (EEG) in veterinary patients with QC. We describe here the EEG changes observed in a dogwith epileptic seizures and in which a QC was identified by magnetic resonance imaging (MRI). We attempted to establish a correlation between the localization of electrical epileptic foci and that of the QC, and to evaluate the value of EEG monitoring in assessing clinical evolution under conservative treatment.

Case presentation

In April 2021, a 7-year-old castrated male Bichon Frise was presented to the Demed Small Animal Practice in Cluj-Napoca, Romania, with a history of three generalized epileptic seizures over the last three weeks. The owner also described episodes of sudden spontaneous changes in behavior (such as head shaking anrubbing of the nose with the forelimbs or to the ground) that started approximately 2 years ago, and their frequency gradually increased. The dog was not under any treatment at the time of presentation.

On clinical examination, the dog was normothermic, and no pathological changes were observed. Neurological examination revealed a normal mental status and behavior, with no cranial nerve deficits. Evaluation of the posture, gait, proprioception, spinal reflexes, and muscle tone was unremarkable. The neuroanatomical localization of the generalized epileptic seizures, documented by video recordings, was assumed to be in the forebrain.

Considering the clinical history and the results of the physical and neurological examination, the list of differential diagnoses included structural brain diseases such as cerebral neoplasm or an inflammatory process of the brain, but metaboliccauses could not be excluded either.

To identify the cause of the symptomatology, a diagnostic protocol including complete blood count (CBC), a standard biochemistry analysis set, pre- and postprandial total serum bile acids, urinalysis, EEG, brain and cervical spine MRIand cerebrospinal fluid (CSF) analysis was recommended. However, the owner only agreed to performing CBC, standard biochemistry analysis, total serum bile acids, urinalysis, and EEG. All routine laboratory investigation results were within the reference ranges.

The first EEG was performed before starting any treatment, with a portable 28- channel equipment (Galileo, EB Neuro S.p.A., Italy), under light sedation with medetomidine (0.5 mg/kg IM). An adapted method of scalp electrode placement was used, based on the 10–20 International System for Humans (8). Subdermal needle electrodes (13x0.4 mm SD52-826-V1, SpesMedica, Italy) were placed to cover the scalp uniformly. A 10-channel referential montage was set up for the recording, consisting of two frontal electrodes (F3 left, F4 right), two central electrodes (C3 left, C4 right), two parietal electrodes (P3 left, P4 right), two occipital electrodes (O1 left, O2 right), two temporal electrodes (T3 left, T4 right), one reference electrode in the vertex (median parietal Pz), and one ground electrode,on the occipital protuberance.

Referential, common average and different bipolarmontages were used for reviewing the digital EEG. The sensitivity was set at 7 μV/mm, the paper speed at 30 mm/s, and the filters were set between 0.5 and 50Hz, with the notch filter on. The setup included one standard electrocardiography(ECG) channel, with an active and a reference electrode, placed on either side ofthe chest. EEG recordings lasted for a minimum of 20 minutes; no activation procedures were performed. In our examinations, the most appropriate view was obtained with the average montage. Other montages are available in supplimentarymaterial.The first spontaneous EEG recording revealed solitary spikes (70–150 μV), and sharp waves mainly on anterior derivations (frontal and central), intercalated with slow waves of high amplitude, every 40–80 s (Fig. 1).

Fig. 1. Aspect of the first electroencephalogram recording in average reference montage.

The recording reveals spikes of varying amplitude (70–150 μV) mainly on frontal derivations,intercalated with slow and high voltage waves. Low-cut filter: 0.5 Hz; high-cut filter: 50 Hz; speed 10 s/page; sensitivity 7 μV/mm; channel C: elctrocardiogram, bipolar on the chest; channels B and D: empty.

These EEG findings, representative for epileptic abnormalities, together with theclear history of generalized epileptic seizures, led to the start of antiepileptic treatment. While the exact cause of the seizures was not identified following the initial investigations accepted by the owner, it was still possible to exclude a metabolic cause. Therefore, a symptomatic antiepileptic treatment was recommended, consisting of phenobarbital in a dose of 2.5 mg/kg every 12 h.

Under this treatment protocol, the dog was seizure-free and had no other symptoms for two months. After this period, the owner returned with the patient, complaining of cluster seizures. Values of hepatic parameters were normal, and the serum phenobarbital concentration was within the therapeutic range. This time,the

owners agreed to perform the brain and cervical spine MRI recommended initially, together with the CSF examination.

MRI images were acquired using a 0.2 Tesla Signa Profile MRI Scanner (GE Healthcare, US), under general anesthesia with isoflurane. The complete brain MRI protocol included the following sequences and weights: T2 sagittal and transversal, fluid-attenuated inversion recovery (FLAIR) and T1 sagittal and transversal.

Contrast substance was also administered. The collection of CSF was not possibleimmediately after the MRI, and the owner did not agree to anesthetizing the patient again. Even in the absence of CSF analysis, an immune-mediated meningoencephalitis was excluded, based on the evolution of the disease, the lack of other neurological symptoms, and the effectiveness of the antiepileptic treatment for two months.

On the MRI scan, a large lesion was visible in the quadrigeminal cistern, identified as an arachnoid cyst based on the following aspects: obvious delimitation from the adjacent parenchymal tissue, clear cystic appearance, hypointense signal on T1 weighted sequences and hyperintense on T2 sequences, but with no capturing of the contrast substance. The cyst was predominantly subtentorial, and displaced thecerebellum ventrocaudally, towards the foramen magnum. No other structural brain lesions were observed on the MRI, including no syringohydromyelia on the cervical segment. The compression exerted by the QC reached 37.5 % in the occipital lobe and 44.4 % in the cerebellar region. The appearance of the MRI and the calculationof the compression percentage (based on (12)) can be seen on Fig. 2A and 2B, respectively.

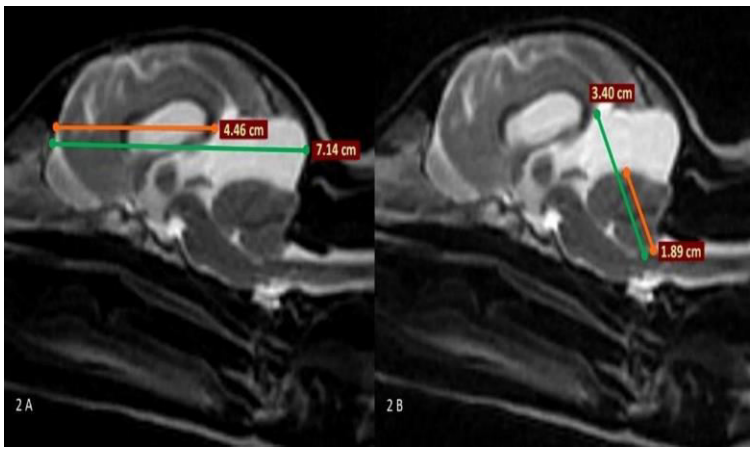


Fig. 2. Measurement of the compression level of the brain parenchyma (2A) and of thecerebellum (2B) on a sagittal T2 weighted magnetic resonance image (9).

2A: The green line (7.14 cm) represents the expected diameter of the brain, while the orange line (4.46 cm) represents the current size of the brain. The compression level of thebrain in this patient was 37.5%.

2B: The green line (3.40 cm) represents the expected diameter of the cerebellum, while theorange line (1.89 cm) represents the current size of the cerebellum. The compression levelof the cerebellum in this patient was 44.4%.

After this diagnosis, the owner decided to continue with conservative treatment, therefore the therapeutic protocol was modified to include additional medication, aslisted in Table 1.

Table 1

Therapeutic protocol

Medication	Dosage and administrationfrequency	Starting	Status
Phenobarbital	2.5 mg/kg, every 12h	Before diagnosis	Ongoing
Prednisolon	0.3 mg/kg, every 24h	Immediately after diagnosis	Ongoing
Omperazol	5 mg/kg, every 24 h	Immediately after diagnosis	Ongoing
Gabapentin	10 mg/kg, every 12 h	Immediately after diagnosis	Ongoing
Acetazolamide	10 mg/kg, every 12 h	Two months after diagnosis	Ongoing

Under the updated treatment, the patient had a good evolution, quality of life improved, seizures were rare in comparison to the initial frequency (one episode in two months); however, the episodes of head shaking persisted, appearing several times a day.

Two more routine EEG registrations were performed at ten-week intervals, underthe same circumstances. The second EEG recording (ten weeks after the initial EEG) revealed solitary spikes (70–150 μV), and sharp waves mainly on the anterior derivations (frontal and central), and more rarely on the parietal derivations. The abnormalities appeared every 30–50s (Fig. 3).

The recording reveals spikes of varying amplitude (70–150 μV), mainly on the anterior derivations (F3, F4, C4). Low-cut filter: 0.5 Hz; high-cut filter: 50 Hz; speed 10 s/page; sensitivity: 7 μV/mm; channel C: electrocardiogram, bipolar on the chest; channel B empty;channel D: left anterior limb.

The third EEG recording (twenty weeks after the initial EEG) revealed epileptic discharges, such as high amplitude spikes, sharp waves (100–150 μV), spike-slowwaves complexes that appeared every 20–40 s, on both hemispheres, on left anterior derivations F3, C3, T3 and on right posterior derivations O2, P4, intercalated with slow waves (Fig. 4).

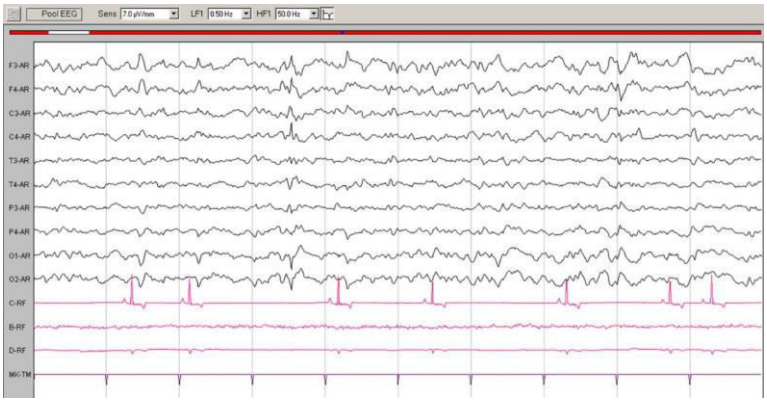


Fig. 3. Aspect of the second electroencephalogram recording in average referencemontage (ten weeks after the first one).

Fig. 4. Aspect of the third electroencephalogram recording in average reference montage(twenty weeks after the first one)

The recording reveals spikes of 100–150 μV amplitude, mainly on left derivations (T3, C3,P3). Low-cut filter: 0.5 Hz; high-cut filter: 50 Hz; speed 10 s/page; sensitivity: 7 μV/mm; channel C: electrocardiogram, bipolar on the chest; channel B empty; channel D: left anterior limb.

EEG images recorded in the longitudinal montage at each timepoint are included in Fig. 5-7.

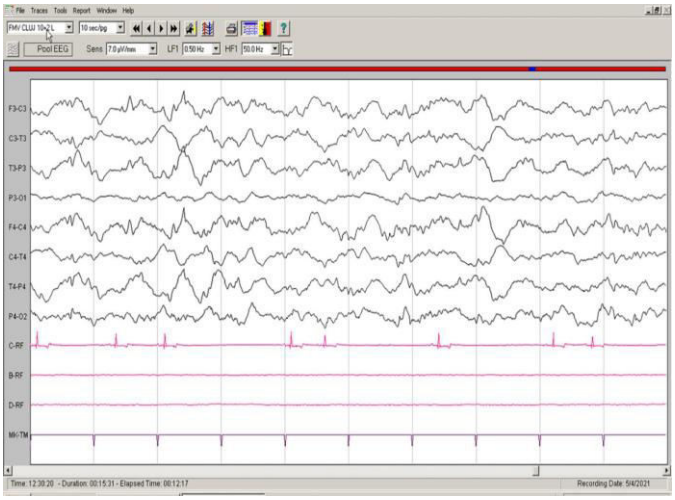


Fig. 5. Aspect of the first electroencephalogram recording in longitudinal montage

Results reveal spikes of varying amplitude (70–150 μV) mainly on F3-C3, F4-C4, intercalated with slow and high voltage waves. Low voltage and low amplitude on P3-O1. Low-cut filter: 0.5 Hz; high-cut filter: 50 Hz; speed 10 s/page; sensitivity 7 μV/mm; channelC: electrocardiogram, bipolar on the chest; channels B and D: empty.

Fig. 6. Aspect of the second electroencephalogram

Second electroencephalogram recorded in longitudinal montage (ten weeks after the first one), revealing spikes of varying amplitude (70–150 µV),mainly on the central and temporal derivations (C3-T3, C4-T4) and F4-C4. Low-cut filter: 0.5Hz; high-cut filter: 50 Hz; speed 10 s/page; sensitivity: 7 µV/mm; channel C: electrocardiogram, bipolar on the chest; channel B empty; channel D: left anterior limb.

Fig. 7. Aspect of the third electroencephalogram

Third electroencephalogram recorded in longitudinal montage (twenty weeks after the first one), revealing spikes of 70-100 µV amplitude, mainlyon left derivations (C3-T3, T3-P3) and frontal bilaterally F3-T3 and F4-T4. Low-cut filter: 0.5Hz; high-cut filter: 50 Hz; speed 10 s/page; sensitivity: 7 µV/mm; channel C: electrocardiogram, bipolar on the chest; channel B empty; channel D: left anterior limb.

We reported here the case of a small-breed, male dog with neurological symptomscaused by a QC. In a large proportion of cases documented in dogs (and humans),QC is purely incidental finding (1, 5, 6, 10, 12, 14, 15). However, when it represents theonly lesion, a QC can be considered responsible for clinical symptoms such as different types of epileptic seizures and cerebello-vestibular dysfunction (4).

For a limited number of canine QC cases reported in the literature, investigations of the relationship between MRI features and the clinical manifestations of the QC have been conducted. Matiasek et al. (12) attempted to establish a connection between the degree of compression exerted by the QC on the cerebellar and/or occipital areas and the likelihood of clinical signs; they found that even with measurable compression of the brain parenchyma, clinical symptoms were not always present, although compression of over 14% in the occipital lobe was associated with neurological manifestations. Our results are in line with this finding: in the case of our patient, the measured occipital compression (37.5%) was high

enough to cause symptoms. Another case report describes repeated MRI analyses of a canine patient with QC, showing that despite long-term conservative treatment, the cyst increased in volume (9).

Despite these interesting and informative attempts of correlating paraclinical data and clinical manifestations of canine QC, the relationship between the localization of the cyst and that of the electric epileptic foci has not yet been investigated; the question of the value of EEG in monitoring the evolution of the QC under conservative treatment also remains open. In human neurology, prolonged surface EEG recordings are still considered essential tools in localizing electric epileptic foci. In one study describing scalp EEG findings in pediatric patients with IAC and concurrent epilepsy, the presence of the epileptic foci was strongly correlated with the location of the cyst (11). However, in most other human cases with IAC reported in the literature, no such relationship between the epileptic foci and the localization of the cyst has been found (2, 7, 13, 18). Our findings are in line with the latter category of studies; in our canine patient, the electrical epileptiform activity appeared mainly on the frontal derivations, at a distance from the localization of the QC confirmed by MRI.

Our study has several limitations. The conclusions are based on one single caninepatient with QC and epileptic seizures, which was followed for a limited time periodof almost one year. Therefore, no information is available on the potential EEG changes earlier in the course of the disease. In humans, other electrophysiological monitoring methods, such as corticography or invasive EEG, have shown greater success in identifying epileptic foci that were correlated with the localization of the IAC (7, 11); however, we only attempted to correlate localization of the QC with that of electrical epileptic foci detected using surface EEG.

Conclusions

In conclusion, in the case described here, surface EEG was not a valuable test forlocalizing the QC or for monitoring the clinical evolution of the canine patient underconservative treatment. However, further data are requires to establish the value ofEEG examination in these patients. To our knowledge, this is the first study that decribes the pathological aspect of EEG in a dog with QC.

Acknowledgements

This scientific paper was carried out in the Demed Clinics Small Animal practice Cluj Napoca, Romania, with the implication of MARAVET, setting up a nationwide referral scheme for dogs with neurological disorders. All authors contributed to the article and approved the submitted version. Medical service were provided by Timea Kiss.

References

1. **Al-Holou, W.N., Yew, A.Y., Boomsaad, Z.E., Garton, H.J., Muraszko, K.M., Maher, C.O.,** Prevalence and natural history of arachnoid cysts in children, Journal of neurosurgery, Pediatrics, 2010, 5, 578-85.
2. **Arroyo, S., Santamaria, J.,** What is the relationship between arachnoid cysts and seizure foci?, Epilepsia, 1997, 38, 1098-102.
3. **Dewey, C.W., Krotscheck, U., Bailey, K.S., Marino, D.J.,** Craniotomy with cystoperitoneal shunting for treatment of intracranial arachnoid cysts in dogs, Veterinary Surgery, 2007, 36, 416-22.
4. **Dewey, C.W.,** APractical Guide to Canine and Feline Neurology, 2nd edition, Ames, Iowa: Wiley- Blackwell; 2008.
5. **Duque, C., Parent, J., Brisson, B., Costa, R., Poma, R.,** Intracranial arachnoid cysts: are they clinically significant?, Journal of Veterinary Internal Medicinem 2005, 19, 772-4.
6. **Duz, B., Kaya, S., Daneyemez, M.,** Surgical management strategies of intracranial arachnoid cysts: A single institution experience of 75 cases, Turkish Neurosurgery, 2012, 22, 591-8.
7. **Gan, Y.C., Connolly, M.B., Steinbok, P.,** Epilepsy associated with a cerebellar arachnoid cyst: seizure control following fenestration of the cyst, Child's Nervous System, 2008, 24, 125-34.
8. **Jasper, H.H.,** Report of the committee on methods of clinical examination in electroencephalography, Electroencephalography and Clinical Neurophysiology, 1958, 10, 370-5.
9. **Kang, M.H., Lee, S.W., Kim, H.J., Woo, E.J., Park, H.M.,** Serial magnetic resonance imaging and long-term medical management of intracranial arachnoid cyst in adog, Pakistan Veterinary Journal, 2014, 34, 417-9.
10. **Kitagawa, M., Kanayama, K., Sakai, T.,** Quadrigeminal cistern arachnoid cyst diagnosed by MRI in five dogs, Australian Veterinary Journal, 2003, 81, 340-3.
11. **Kushen, M.C., Frim, D.,** Placement of subdural electrode grids for seizure focus localization in patients with a large arachnoid cyst, Technical note, Neurosurgery Focus, 2007, 15, E5.
12. **Matiasek, L.A., Platt, S.R., Shaw, S., Dennis, R.,** Clinical and magnetic resonanceimaging characteristics of quadrigeminal cysts in dogs, Journal of Veterinary Internal Medicine, 2007, 21, 1021-6.
13. **Rocco, C., Tamburrini, G.,** The Causes of Epilepsy: Common and Uncommon Causesin Adults and Children, Cambridge University Press, 2011.
14. **Saito, M., Olby, N.J., Spaulding, K.,** Identification of arachnoid cysts in the quadrigeminal cistern using ultrasonography, Veterinary Radiology & Ultrasound, 2001, 42, 435-9.
15. **Vernau, K.M., Kortz, G.D., Koblik, P.D., LeCouteur, R.A., Bailey, C.S., Pedroia, V.,** Magnetic resonance imaging and computed tomography characteristics of intracranial intra-arachnoid cysts in 6 dogs, Veterinary

Radiology & Ultrasound, 1997, 38, 171-6.
16. **Vernau, K.M., LeCouteur, R.A., Sturges, B.K., Samii, V., Higgins, R.J., Koblik, P.D., Vernau, W.,** Intracranial intra-arachnoid cyst with intracystic hemorrhage in two dogs,Veterinary Radiology & Ultrasound. 2002, 43, 449-54.
17. **Wang, C., Han, G., You, C., Liu, C., Wang, J., Xiong, Y.,** Individual surgical treatment of intracranial arachnoid cyst in pediatric patients, Neurology India, 2013, 61, 400-5.
18. **Yalçin, A.D., Oncel, C., Kaymaz, A., Kuloğlu, N., Forta, H.,** Evidence against association between arachnoid cysts and epilepsy, Epilepsy Research, 2002, 49, 255-60.

THE INFLUENCE OF BLOOD GAS ANALYSIS IN THE PROGNOSIS OF GASTROINTESTINAL STASIS IN THE DOMESTIC RABBIT (*ORYCTOLAGUS CUNICULUS*)

DOBOȘI A.A., BEL L.V., TURCU M.C., BUMBU M.G., PUSTA D.

University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca,
Faculty of Veterinary Medicine, 400372,
Calea Mănăștur No. 3-5, Cluj-Napoca, Romania
E-mail: anca.dobosi@student.usamvcluj.ro

Summary

The aim of this study was to perform blood gas analysis both in rabbits diagnosed with gastrointestinal stasis and clinically healthy rabbits, to compare the results and evaluate blood gases values influence on the prognosis. The study included 7 domestic rabbits diagnosed with gastrointestinal stasis and 5 clinically healthy domestic rabbits. Medical treatment was instituted in all patients with gastrointestinal stasis at the time of hospitalization. 2 of the rabbits from the gastrointestinal stasis group died. The analyzed parameters were: body temperature, blood pH, pCO_2, HCO_3, BE_{ecf}, Na^+, K^+, Cl^-, blood glucose and lactate. According to the results of this study, low body temperature may be considered a possible negative prognosis factor in rabbits with gastrointestinal stasis. The evaluation of blood lactate did not reveal statistically significant differences between the two groups, but its low value at hospitalization may be of importance for determining the prognosis. Both the small number of patients and the number of blood samples can be factors that limit results regarding the evaluation of lactate influence on the prognosis of the gastrointestinal patient, therefore a larger number of patients would be needed for better evaluation.

Keywords: domestic rabbit, *Oryctolagus cuniculus*, gastrointestinal stasis, blood gas.

Gastrointestinal stasis is a very common syndrome in domestic rabbits, in which feeding and defecation are stopped due to the slowing of gastrointestinal motility. This is considered an emergency and therefore the institution of therapy right away is of the utmost importance (10, 21).

The causes of this syndrome are various and hard to list, because any underlying disease or stress on the rabbit can trigger the entire pathological process. Most often the etiology involves an improper diet, genetic factors, stress factors or secondary diseases such as dental disease, neoplasias, parasitary or infectious diseases (10, 17).

In regards to the clinical symptoms, these usually start with the rabbit having a decreased appetite until total stop of feeding and defecation. An accumulation of gas all over the gastrointestinal tract leads to pain in the abdominal area, manifested by lack of exercise, teeth grinding or a hunched position. On clinical examination the temperature can be low or in normal limits, the abdomen is usually distended, painful and with few or no gut sounds present. For

further diagnostic identification other exams such as X-ray and abdominal ultrasound can be used (20, 21).

The therapy used in this syndrome has five general points that need to be fulfilled. These include rehydration of the patient via intravenous or subcutaneous route, analgesia with non-steroidal anti-inflammatory drugs or/and opioids, stimulation of gastrointestinal motility with prokinetic agents, resumption of nutrition through syringe feeding and, in the case of dysbiosis, administration of antibacterial agents. Other points that can be taken into consideration, depending on the severity of the condition of each patient, are providing heat through incubators, air heaters or electric pillows, ensuring a stress-free environment for the patient and administering anti-flatulence medication. The duration of treatment varies in each patient, most often the symptomatology is improved after only 24-48h from the debut, but it usually lasts for at least 3 to 5 days. In the case of severe cases the patients may die (17, 20, 21).

This study was performed in order to make a comparison of blood gas analysis of ill rabbits and clinically healthy rabbits and to evaluate the influence of these values on the prognosis of gastrointestinal stasis.

Materials and methods

The population of this study consisted of a group of 7 rabbits diagnosed with gastrointestinal stasis, of which 4 males and 3 females, aged between 1 and 4 years old, weighing between 1.5 kg and 2.8 kg. This group underwent two blood gas analyzes, at the moment of hospitalization and at return to feeding and defecation, except for the 2 patients that died, having had only one blood sampling performed. The control group consisted of 5 clinically healthy rabbits, of which 3 males and 2 females, aged between 6 months and 2 years old, weighing between 815 g and 2 kg. This group underwent a single blood gas analysis at the moment of clinical examination. All the rabbits included in this research were presented at the Clinic of New Companion Animals of the Faculty of Veterinary Medicine Cluj-Napoca, Romania.

Approximately 0.2 ml blood was sampled from each rabbit from the lateral saphenous vein using 2 ml heparinized syringes and 23G needles. In addition, temperature of the rabbits was assessed at each blood draw using an electronic rectal thermometer.

Blood gas analysis was performed using the Stat Profile Prime Plus VET Critical Care Analyzer, the analyzed parameters were: blood pH, pCO_2, HCO_3, BE_{ecf}, Na^+, K^+, Cl^-, glucose and lactate.

Results and discussions

After the institution of treatment on the group diagnosed with gastrointestinal stasis, 2 of the 7 rabbits died before being able to perform the

second blood gas analysis. The other 5 rabbits were considered cured after treatment. In Table 1 and Table 2 the results of all the blood gas analyzes performed on the two groups are presented.

Table 1

Results of the blood gas analyzes of the group diagnosed with gastrointestinal stasis

Parameter	Value interval at hospitalization	Arithmetic mean ± SD	Number of patients	Value interval at repetition	Arithmetic mean ± SD	Number of patients
Body temperature (°C)	37.7 – 39.1	38.6 ± 0.5	7	39.2 – 39.7	39.32 ± 0.3	5
Blood pH	7.20 – 7.42	7.30 ± 0.07	7	7.20 – 7.43	7.33 ± 0.09	5
pCO_2	11.9 – 39.3	29.9 ± 9.33	7	24.8 – 58.3	39.3 ± 15.1	5
HCO_3 (mEq/L)	4.7 – 21.6	16.3 ± 6.05	7	14.4 – 28.6	20.54 ± 5.56	5
BE_{ecf} (mmol/L)	-23.5 - -3.1	-10.2 ± 6.95	7	-14 - +2.6	-5.8 ± 5.97	5
Na^+ (mEq/L)	138.7 – 149.4	142.9 ± 3.79	7	141.4 – 148.4	144.5 ± 2.64	5
K^+ (mEq/L)	3.42 – 6.68	4.87 ± 1.09	7	3.89 – 5.14	4.64 ± 4.64	5
Cl^- (mEq/L)	108 – 118.7	112.8 ± 3.85	7	107.3 – 117.6	111.46 ± 3.77	5
Glucose (mg/dL)	96 - 255	140.4 ± 53.55	7	77 - 144	118.2 ± 24.75	5
Lactate (mmol/L)	3.7 – 17.9	8.6 ± 4.89	7	2.4 – 14.2	8.5 ± 5.00	5

SD – Standard Deviation

Table 2

Results of the blood gas analyzes of the control group

Parameter	Value interval of control group	Arithmetic mean ± SD
Body temperature (°C)	38.2 – 39.3	38.64 ± 0.45
Blood pH	7.22 – 7.42	7.32 ± 0.08
pCO_2	33.1 – 43.5	37.96 ± 4.71
HCO_3 (mEq/L)	18 - 22.8	20.08 ± 2.02
BE_{ecf} (mmol/L)	-9.9 - -1.8	- 6.1 ± 3.19
Na^+ (mEq/L)	140.4 – 144.5	142.54 ± 1.71
K^+ (mEq/L)	4.66 – 6.69	5.60 ± 0.89
Cl^- (mEq/L)	102.1 - 106.1	104.4 ± 1.62
Glucose (mg/dL)	133 - 215	161 ± 35.06
Lactate (mmol/L)	12.2 - 21	16.5 ± 3.51

SD – Standard Deviation

All parameters evaluated in this study and reference ranges cited by different authors can be seen in Table 3.

Body temperature, one of the parameters examined, can physiologically rise in rabbits in stressful situations and decrease in case of an underlying disease. Some authors think that assessing body temperature in rabbits is of low clinical value because of both the risk of producing lesions of the rectal mucosa and the stress involved (22, 23).

In gastrointestinal stasis many studies showed that body temperature tends to be below reference values. In 2016, Di Girolamo et al. (9) considered a temperature ≤ 37.9°C in rabbits to be hypothermia, while Oparil et al. (19), in 2019, defined hypothermia in rabbits as a body temperature ≤ 36.6°C. Moreover, Di Girolamo et al. (9) discovered that with every 1°C drop in body temperature from the moment of hospitalization the mortality chances were doubling.

In this research, one patient presented a body temperature lower than the reference values, namely 37.7°C, this also being one of the patients that died, suggesting that hypothermia may be an important indicator of a poor prognosis.

Blood pH, pCO_2 (partial pressure of carbon dioxide), HCO_3 (bicarbonate) and BE_{ecf} (base excess in the extracellular fluid compartment) need to be evaluated together for a clinically significant value. By interpreting the values of

these parameters we can establish if the patient suffers from a metabolic acidosis or alkalosis, respiratory or non-respiratory, with a compensatory response or not.

Table 3

Reference intervals for the parameters evaluated in this study and by other authors

Parameter	Reference interval in the present study	Ardiaca et al. (2), 2013	Gallego (11), 2017	Carpenter (8), 2017	Wesche (24), 2014	Melillo (16), 2007
Body temperature (°C)	38.2 – 39.3	-	37.4 – 39.6	38.5 - 40	-	-
Blood pH	7.22 – 7.42	7.25 – 7.53	7.23 – 7.56	-	-	-
pCO2 (mmHg)	33.1 – 43.5	28.9 – 52.9	28.5 – 50.7	-	-	-
HCO3 (mEq/L)	18 - 22.8	17 - 32.5	15.8 – 30.2	16.2 – 31.8	16 - 32	-
BEecf (mmol/L)	-9.9 - -1.8	-10 - +8	-8.8 - +5.7	-	-	-
Na^+ (mEq/L)	140.4 – 144.5	136 – 147	139 – 149	138 - 155	130 - 155	138 – 150
K^+ (mEq/L)	4.66 – 6.69	3.4 – 5.7	3.8 – 6.1	3.5 - 7	3.3 – 5.7	3.5 – 6.9
Cl^- (mEq/L)	102.1 - 106.1	93 - 113	96 - 113	92 - 112	92 - 120	-
Glucose (mg/dL)	133 - 215	106 - 205	112 - 231	75 - 150	99 - 148	75 - 155
Lactate (mmol/L)	12.2 - 21	2.1 – 15.2*	2 - 10	-	-	-

*Ardiaca et al. (3)

Firstly, blood pH is assessed by considering 7.4 the mean normal value in rabbits, so when the pH is < 7.4 acidemia is present and when the pH is > 7.4 alkalemia is present. Then pCO_2, the respiratory component, is analyzed in order to evaluate the type of acid-base imbalance present. For rabbits the reference value of 40 mmHg is taken into account for pCO_2, so this is usually higher when there is hypoventilation involved, accompanied by a low blood pH, and it is lower following hyperventilation, associated with a high blood pH. Lastly, HCO_3 and BE_{ecf} are evaluated as the non-respiratory component of the acid-base balance. Higher values than the reference range of these two parameters suggest a metabolic alkalemia, while values below reference range suggest a metabolic acidosis (2, 5).

In gastrointestinal stasis in the rabbit, as well as in pyloric obstruction, the usual case is of acid accumulation in the stomach and a continuous secretion of alkaline secretions in the intestines, which can lead to a hypochloremic alkalosis. Metabolic acidosis usually results from a loss of bicarbonate either through

obstructions at a lower level of the gut segment or through diarrhea. Depending on the case, the patient can suffer a metabolic acidosis, metabolic alkalosis or a mixed disorder (2). A study conducted by Brezina et al. (6) on rabbits with gastrointestinal stasis indicated that the majority presented a metabolic acidosis and also that the values of HCO_3 and BE_{ecf} were much lower the longer the time until the gastrointestinal stasis or gastric dilation was treated.

The values of blood pH, pCO_2, HCO_3 and BE_{ecf} from the rabbits in this study showed a simultaneous decrease compared to the reference ranges in one patient, who subsequently died, suggesting a metabolic acidosis with a respiratory compensation. At the first blood gas analysis, 2 out of 7 rabbits had a simultaneous decrease in pCO_2, HCO_3, BE_{ecf} and a blood pH close to the inferior limit of the reference interval, also suggesting the possible presence of a metabolic acidosis with respiratory compensation. One of these two patients subsequently died and the other showed approximately the same values at repetition of the blood gas analysis. The other values of these parameters that were singularly high or low have no significant clinical value for a conclusion to be made.

Na^+ (sodium), another parameter assessed in this study, is an ion responsible for plasma osmolality. Low values of this parameter in the blood, called hyponatremia, are usually characterized by neurologic symptoms with a possible formation of cerebral edema. Based on tonicity, hyponatremia can be of two types: true hyponatremia (hypotonic hyponatremia) and pseudohyponatremia (isotonic or hypertonic hyponatremia). Hypotonic hyponatremia is usually associated with low plasma tonicity, isotonic hyponatremia with hyperglycemia, hyperproteinemia and hyperlipidaemia and hypertonic hyponatremia with hyperglycemia and manitol administration. Pseudohyponatremia has no actual consequence on the patient and therefore does not need to be treated, hence the importance of identifying it. On the other hand, high levels of sodium in the blood, called hypernatremia, is less often found and is caused by the loss of hypotonic fluids and a water deficit (2, 7).

In rabbits pseudohyponatremia usually occurs as a consequence of hyperglycemia, because Na^+ drops in response to high levels of glucose in the blood as a mechanism to mantain plasma tonicity (2).The results of this research show that 2 of the 7 rabbits had a lower sodium than the reference range at first blood gas analysis, but only one of them had a simultaneous hyperglycemia, which suggests a pseudohyponatremia. Hypernatremia could be seen in 3 of the 7 rabbits at first blood gas analysis, respectively in 2 out of 5 rabbits at analysis repetition, and can be interpreted as a result to dehydration.

K^+ (potassium) is an essential electrolite in maintaining membrane potential. Hypokalemia in rabbits can appear as a result of poor intake, loss of fluids through the gastrointestinal level or renal insufficiency as well as from a rise in cathecolamines in stress. On the other side, hyperkalemia is usually the consequence of acute renal insufficiency, lower urinary tract obstruction or severe tissue distruction that spreads potassium in the extracellular compartment (2, 16).

In this study potassium was lower than the reference range in 3 rabbits at both analyzes, most likely because of a poor intake or stress.

Cl^- (chloride), the last electrolite discussed in this study, together with sodium is responsible for maintaining plasma osmolality, acid-base balance and electroneutrality of body fluids (4). In rabbits this parameter is physiologically lower than in other species. Chloremia appears to be directly propotional to natremia, so that most rabbits with hyponatremia also show a hypochloremia (2). The study conducted by Brezina et al. (6) showed that rabbits presented hypochloremia in metabolic alkalosis. The other situation, namely hyperchloremia, also present at all the patients in this study at both analyzes, usually happens as a compensatory mechanism to a bicarbonate deficit. Because this is pretty unusual and the number of rabbits in this study was quite low, these results can't be considered to have a clinical value.

Blood glucose is a very important parameter that needs to be assessed. The concentration of glucose in the blood is influenced by many aspects such as the food type in the gastrointestinal tract, gluconeogenesis, glycogenolysis and the absorption of glucose in the muscular tissue and other tissues. The glucose metabolism is also regulated with the help of hormones like insulin, glucagon, glucocorticoids and cathecolamines (12).

Hypoglycemia can have many causes, such as insulin-secreting tumors, sepsis, liver disease, mucoid enteropathy, endocrine diseases, excessive physical effort or an insufficient glucose absorbtion by the gastrointestinal tract. It can also be seen in anorexic rabbits, suggesting that they use adipose tissue and are at risk of developing hepatic lipidosis (12, 16).

Hyperglycemia is usually seen in diabetes, but can also appear in other underlying diseases or stress. Because of the way the digestive tract of rabbits is designed, namely that their stomach is never empty, it is impossible to get a blood glucose analysis with a black diet beforehand, as you do for other species. Rabbits constantly ingest cecotrophs and it was demonstrated that even a 4 day starvation didn't register lower blood glucose levels (12, 14).

The study of Harcourt-Brown and Harcourt-Brown (12) in 2012 showed that stress is a very important factor contributing to hyperglycemia in rabbits. Even in clinically healthy rabbits blood glucose tends to be at higher levels than reference ranges because of this, which makes it hard to assess it in cases of actual diseases. A small number of rabbits with gastrointestinal stasis presented hypoglycemia, most likely because of a poor food intake and a slowed gut motility that doesn't allow an appropriate supply with nutrients. It was also observed that blood glucose levels were much higher in rabbits with intestinal obstruction compared to those with gastrointestinal stasis, concluding that the degree of hyperglycemia can be a predictive factor in life-threatening diseases of rabbits (12).

In the present study no significant changes of blood glucose were seen in the gastrointestinal stasis group. A single rabbit showed a higher value than the reference interval at first analysis, most likely as a response to stress and pain.

Some of the rabbits had hypoglycemia at both blood gas analyzes, probably due to the reduced food intake.

Blood lactate, the final parameter evaluated in this study, is the product of glucose metabolism, most of it being produced though anaerobic glycolysis. When a high blood lactate concentration is accompanied by a low blood pH it is called lactic acidosis, this being the result of a tissue hypoperfusion and hypoxia in shock, severe anemia, sepsis, respiratory distress or in hypermetabolic states (1, 15).

Several studies showed that a high blood lactate is an indicator for a poor prognosis in horses (13), dogs (18) and even humans (1). This is not the case in rabbits, however, where the blood lactate is physiologically at high levels because of their particular way of digestion. Determining this parameter in this species can be of importance in evaluating tissue perfusion and the integrity of the gastrointestinal barrier as well as in monitoring the response to treatment and establishing a prognosis (3, 15).

The study of Hupfeld (13) in 2009 showed no significant changes of blood lactate values between healthy and ill rabbits, but some higher values could still be observed in the ill rabbits compared to the healthy ones. Another study done by Ardiaca et al. (3) in 2016 revealed that rabbits with a lower blood lactate value had a poorer prognosis than those with a higher value. Moreover, a rise of 3.3 mmol/L blood lactate in 48 hours from the moment of hospitalization was associated with a favorable outcome. The authors concluded that blood lactate is a good parameter for treatment monitorization and prognosis evaluation (3).

The blood lactate assessed in this study showed variations. The values were lower than the reference interval in 5 out of 7 rabbits at first analysis, respectively in 2 out of 5 rabbits at second analysis. From these results we can also conclude that low lactate values are suggestive for a poorer prognosis contrary to other species, but the number of patients in this research can’t give for a statistically relevant conclusion.

Conclusions

Blood gas analysis of the rabbit patient must be interpreted in a clinical context.

Body temperature can be used in the prognosis evaluation of rabbits diagnosed with gastrointestinal stasis, the lower it is at the time of hospitalization the poorer the prognosis.

In terms of blood lactate, even if the differences between the two groups weren’t statistically significant, it was lower at the initial moment of the syndrome, which could have a value for determining the prognosis.

Further studies are necessary in order to observe the modifications of blood gas depending on the localization of the gastrointestinal pathology.

Gastrointestinal stasis is an emergency, early therapy institution being of the utmost importance in order to prevent irreversible lesions and death of the rabbit patient.

Acknowledgement

This study was performed with the help of the entire Clinic of New Companion Animals (NAC) of the Faculty of Veterinary Medicine, Cluj-Napoca, Romania.

References

1. **Allen, S.E., Holm, J.L.,** Lactate: physiology and clinical utility, Journal of Veterinary Emergency and Critical Care, 2008, 18, 2, 123-132.
2. **Ardiaca, M., Bonvehi, C., Montesinos, A.,** Point-of-care blood gas and electrolyte analysis in rabbits, Veterinary Clinics: Exotic Animal Practice, 2013, 16, 1, 175-195.
3. **Ardiaca, M., Dias, S., Montesinos, A., Bonvehi, C., Barrera, S., Cuesta, M.,** Plasmatic l-lactate in pet rabbits: association with morbidity and mortality at 14 days, Veterinary clinical pathology, 2016, 45, 1, 116-123.
4. **Bandak, G., Kashani, K.B.,** Chloride in intensive care units: a key electrolyte, F1000 Research, 2017, 6.
5. **Bateman, S.W.,** Making sense of blood gas results, Veterinary Clinics of North America: Small Animal Practice, 2008, 38, 3, 543-557.
6. **Brezina, T., Fehr, M., Neumüller, M., Thöle, M.,** Acid-base-balance status and blood gas analysis in rabbits with gastric stasis and gastric dilation, Journal of Exotic Pet Medicine, 2020, 32, 18-26.
7. **Bonvehi, C., Ardiaca, M., Barrera, S., Cuesta, M., Montesinos, A.,** Prevalence and types of hyponatraemia, its relationship with hyperglycaemia and mortality in ill pet rabbits, Veterinary Record, 2014, 174, 22, 554.
8. **Carpenter, J.W., Marion, C.,** Exotic Animal Formulary, Elsevier Health Sciences, St. Louis, Missouri, 2017.
9. **Di Girolamo, N., Toth, G., Selleri, P.,** Prognostic value of rectal temperature at hospital admission in client-owned rabbits, Journal of the American Veterinary Medical Association, 2016, 248, 3, 288-297.
10. **Fisher, P.G.,** GI Disease of Rabbits and Ferrets, 2015.
11. **Gallego, M.,** Laboratory reference intervals for systolic blood pressure, rectal temperature, haematology, biochemistry and venous blood gas and electrolytes in healthy pet rabbits, Open Veterinary Journal, 2017, 7, 3, 203-207.
12. **Harcourt-Brown, F.M., Harcourt-Brown, S.F.,** Clinical value of blood glucose measurement in pet rabbits, Veterinary Record, 2012, 170, 26, 674.
13. **Hupfeld, C.,** Lactate concentration in blood of healthy and ill rabbits, Tierärztliche Praxis Ausgabe K: Kleintiere/Heimtiere, 2009, 37, 04, 244-249.
14. **Kozma, C., Macklin, W., Cummins, L.M., Mauer, R.,** Anatomy, physiology and biochemistry of the rabbit, The Biology of the Laboratory Rabbit, 1974, 12, 1, 59-64.

15. **Langlois, I., Planché, A., Boysen, S.R., Abeysekara, S., Zello, G.A.,** Blood concentrations of d-and l-lactate in healthy rabbits, Journal of Small Animal Practice, 2014, 55, 9, 451-456.
16. **Melillo, A.,** Rabbit clinical pathology, Journal of Exotic Pet Medicine, 2007, 16, 3, 135-145.
17. **Mitchell, M., Tully Jr., T.N.,** Current Therapy in Exotic Pet Practice, Elsevier Health Sciences, St. Louis, Missouri, 2016.
18. **Mooney, E., Raw, C., Hughes, D.,** Plasma lactate concentration as a prognostic biomarker in dogs with gastric dilation and volvulus, Topics in Companion Animal Medicine, 2014, 29, 3, 71-76.
19. **Oparil, K.M., Gladden, J.N., Babyak, J.M., Lambert, C., Graham, J.E.,** Clinical characteristics and short-term outcomes for rabbits with signs of gastrointestinal tract dysfunction: 117 cases (2014–2016), Journal of the American Veterinary Medical Association, 2019, 255, 7, 837-845.
20. **Prebble, J.,** Gastrointestinal stasis and obstructive Ileus in the rabbit, Veterinary Nurse, 2012, 3, 6, 366-372.
21. **Quesenberry, K., Carpenter, J.W.,** Ferrets, Rabbits and Rodents-E-Book: Clinical Medicine and Surgery, Elsevier Health Sciences, St. Louis, Missouri, 2011.
22. **Richardson, V.,** Rabbits: health, husbandry and diseases, Oxford, England: Blackwell Science, 2000.
23. **Varga, M.,** Textbook of Rabbit Medicine (2nd edn), Butterworth-Heinemann, Oxford, 2014.
24. **Wesche, P.,** Clinical pathology, British Small Animal Veterinary Association, Gloucester, 2014.

THE INCIDENCE OF KETOSIS IN 4 DAIRY FARMS FROM SATU MARE COUNTY

GIURGIU O., BEREAN D.I., CIUPE S., BOGDAN L. M., IONESCU A.D., GHERMAN L.M.

University of Agricultural Sciences and Veterinary Medicine from Cluj-Napoca
Faculty of Veterinary Medicine, 400372, Calea Mănăştur No. 3-5, Romania
E-mail: ovidiu.giurgiu@usamvcluj.ro

Summary

A study was carried out in 4 farms with a total of 320 cattle in the first 80 days after calving, from each animal included in the study was collected a milk sample from which was determined the concentration of BHB (betahydroxybutyrate), fat and protein. Cattle were grouped according to season, breed and farm of origin, each variable being reported at the BHB level in milk. The highest level of BHB was found in spring (28.87%) with a significant decrease for the following seasons until winter where the level of BHB was the lowest. The prevalence of ketosis for all animals studied was 17.82% relative to BHB concentration in milk. Compared to the prevalence for each farm, it was established that two farms (A and B) have a higher percentage of animals affected by ketosis, 19.64%; 18.95%, compared to farms C and D where the percentage of affected animals is significantly lower represented by 2.22% and 3.03%. The breed most affected by ketosis was the German spotted cattle (Baltata Germana) followed by the Romanian spotted cattle (Baltata Romaneasca) and Red Holstein. The fat in cow's milk with higher beta-hydroxybutyrate levels was higher by 8.90% and protein by 1.76% lower.

Key words: ketosis, incidence, cows.

Ketosis, either presented at a subclinical or clinical level, is a common metabolic condition in the modern high producing dairy cow and has been associated with many fresh cow diseases (7). Ketosis is a common metabolic disorder in dairy cattle, which occurs 2-4 weeks after parturition, characterized by increased concentrations of ketones, such as betahydroxybutyrate (BHBA), acetoacetate (AcAc), and acetone in milk, urine and blood (18). More screening protocols to diagnose HYK involve and the testing of cows during the first 2 weeks of lactation because of the elevated prevalence of HYK reported during this period (16, 19, 20).

The imbalance between energy requirements for milk production and energy intake through feed causes a negative energy balance, which results in metabolic conditions such as hyperketonemia (21). This one is associated with reduced milk production (6), reduced reproductive performance (22), and increased risk of displaced abomasum (6, 10) and clinical ketosis (17).

Only few papers have used milk BHB concentration to detect HYK and, considering the limitations of strip tests and MIRS prediction models, there is no clear cut-off point. Ranges of milk BHB concentrations to classify cows with suspect HYK (0.15 to 0.19 mmol/l) or positive HYK ($\geqslant$0.20 mmol/l) have been recently proposed

by Koeck et al. (2014) and Santschi et al. (16). On the other hand, Lee et al. (11) considered cows as affected by SCK with milk BHB concentration between 0.01 and 0.20 mmol/l and affected by CK with milk BHB concentration ⩾0.20 mmol/l (2).

Factors that should be considered for interpreting HYK occurrence are calving season, breed and herd management. Authors generally agreed to identify spring as the season with greater prevalence of HYK, whereas contrasting results have been reported for late autumn and winter or summer. Concerning breed, higher overall HYK prevalence in Jersey (19%) than Holstein cows (14%), with values that ranged from 11.4% to 25% in Jersey herds and 0% to 28% in Holstein herds, has been observed by Chandler et al. (5). Prevalence of SCK can vary between farms; reported prevalence rates range from 8.9 to 43% in the first 2 month of lactation (1).

A negative association between the increase of herd size and HYK prevalence has been reported (3) as bigger herds usually implemented strategies such as grouping cows based on milk production to better meet nutritional requirements. Berge and Vertenten (3) also observed a lower prevalence of HYK in systems with cubicles, cubicles and yards, or tie-up bars rather than in systems with straw yards and a slightly greater frequency in systems in which cows were on pastures rather than housed indoor.

Moreover a lower prevalence of HYK was reported in herds feeding forage and concentrate separately or total mixed ration compared with herds using partial mixed ration. Mixed ration refers to cows fed a total mixed ration between grazing periods. These differences in prevalence might be due to the fact that farmers cannot easily control animals in terms of nutritional level and health status when they are on pasture or housed in straw yards (2).

Hyperketonemia has been associated with higher milk fat content, fat-to-protein ratio (F:P) and energy corrected milk and lower protein, lactose and urea nitrogen in milk (8). An increment in fat content between 2.4% (21) and 23.9% (16) has been reported in hyperketonemic compared with healthy cows. Growth of ketone bodies negatively affects milk protein content; hyperketonemic animals produced milk with 0.3% (16) to 11.6% (5) less protein compared with healthy animals.

Some studies have reported milk losses among HYK+ cows ranging from 1.2 to 2.3 kg/d at the first milk test compared with HYK− cows (4, 12, 16). Others have shown an increase in milk yield from 1.2 to 2.4 kg/d during the same period when comparing HYK+ and HYK− cows (13, 14, 21).

Materials and methods

The animals come from four farms, in the study being included a number of 320 cows in the first 80 days postpartum, their distribution according to the farm of origin, breed and the season in which the birth took place. Of the four farms included in the study, three are located in Satu Mare County and one located in Bistrița Năsăud County, in a proportion close to the maximum limit, they have only one farm

breed, so in order to have a homogeneity in terms of breed were excluded from the study cows whose genetic basis is not predominant on the farm.

Following the collection of data from the breeding registers, we established the animals whose gestation is at the end and the parturition will take place in the next week, they were monitored both in the antepartum and especially postpartum period where the clinical evolution of the puerperium clinical development of ketosis.

In order not to disturb the degree of comfort of the animals included in the study, we resorted to the determination of milk ketone bodies through the genetic improvement program of cattle. Samples were collected once a month at a set date, they were collected from each individual cow in sterile containers and added a preserve for their maintenance.

After collecting all the results, we divided the animals studied into three categories: negative-BHB concentration <0.15mMol / l; suspect - BHB concentration 0.15-0.19 mMol / l; positive- BHB concentration> 0.20mMol / l, these were reported in season, breed and farm of origin.

Results and discussions

Betahydroxybutyrate concentrations were analyzed from the perspective of the season, so in the spring months the highest percentage of animals in the positive category was included, and the percentage will decrease in the following seasons to the lowest percentage recorded in winter (Table 1).

Table 1

Distribution of animals according to season, breed and farm of origin

No Crt	Farm of origin	No. of animals	No. Crt	Season	No. of animals	No. Crt	Breed	No. of animals
1	A	56	1	Spring	97	1	Bălțată românească	101
2	B	153	2	Summer	54	2	Bălțată germana	153
3	C	45	3	Autumn	96	3	Red Holstein	66
4	D	66	4	Winter	73		Total	320
	Total	320		Total	320			

According to data obtained in the spring season, the risk of developing ketosis is the highest and moderate in the summer season, reported in T. Vanholder et al. (21) where it suggests that the risk is high in all seasons except in late autumn and early winter. Another study admits that cows that calved from April till June had the highest rates of ketosis, with rates about two times higher than cows that calved

in July through September, cows that calved in January to March had a 1.5 times higher risk of ketosis compared to cows that gave birth in July to September (3).

The results (Table 2) obtained are close to the data reported by other authors, being differences in terms of the winter season where in this study we obtained the lowest values of ketosis compared to other studies where the values were higher, possible differences are possible due to many seasonal factors specific to each region, countries, farm, for which additional research is needed in terms of primarily the food administered, air temperature, humidity, maintenance system etc.

Table 2

Distribution according to season reported at the level of beta-hydroxybutyrate

	Category	Negative	Suspected	Positive
SEASON	Spring	65.98%	5.15%	28.87%
	Summer	77.78%	3.70%	18.52%
	Autumn	92.71%	3.13%	4.17%
	Winter	93.15%	5.48%	1.37%

Regarding the prevalence of ketosis, reports were made between 11.2 to 36.6% (19), in another study the prevalence of HYK was 21.5% in wk1 and 16.6% in week 2, in addition, 8.0% of the cows were diagnosed with HYK in both weeks (15). Santschi et al. (16) reports an incidence between 25 and 60%, according to the data obtained the prevalence was 17.82% compared to BHB in milk, being included in this percentage cows considered positive and suspicious. Of these animals whose BHB value was increased by 37 animals, representing 11.56% of the total animals studied, clinically showed ketosis.

The origin of the cows studied led to the hypothesis that independent animal factors such as the type of feed used, the way they are administered and the system of maintenance in shelters could be involved in the incidence of ketosis, so the percentage obtained presented in Table 3 indicates a significant increase in two farms. This increase can be attributed to the factors mentioned above, but a certain causality is established only after a thorough analysis of all factors.

The classification of purebred animals, compared to the BHB level in milk, led to the identification of the German spotted breed as the most affected by ketosis, results included in Table 4. Chandler et al. (5) report that the prevalence is higher in the Jersey breed (19%) than in the Holstein breed (14%). In terms of data obtained, they can be compared in terms of prevalence as close to the percentage obtained by other authors but in different races. Indeed, the significance from the point of view of the breed is not solid, in order to establish with certainty the susceptibility of the

breed to ketosis, it is necessary to maintain the same conditions and to feed the cattle with the same assortment of fodder.

Table 3

Percentage of animals reported to the farm of origin and BHB level

	Percent/farm			
	A	B	C	D
Negative	76.79	75.82	93.33	93.94
Suspected	3.57	5.23	4.44	3.03
Positives	19.64	18.95	2.22	3.03

In general, cows affected by ketosis have a higher fat content and a lower protein content (2), so following the determination of milk fat we found that cows in the suspicious and positive group had a percentage fat by 8.90% higher than cows in the negative group. Regarded in milk protein, cows in the positive and suspicious group had a percentage of 1.76% lower than the negative group.

Table 4

Percentage of animals relative to BHB breed and level

Percent/race			
	Baltata Germana	Bălțata Romaneasca	Red Holstein
Negative	75.82	84.16	93.94
Suspected	5.23	3.96	3.03
Positives	18.95	11.88	3.03

Conclusions

The results obtained in the four farms indicate that ketosis has a fairly high prevalence with different values depending on the season, breed and management factors in each farm. Reporting the production to the development of ketosis reflects directly in the farm economy, so due to the factors with a possible causality in the development of the pathology it is necessary to implement inter management systems for each farm, comprising first of all a balanced nutrition with animals. A real help in assessing the prevalence of ketosis is the determination of ketone bodies in milk because stressors on cows are minimized. In order to reduce the prevalence as much as possible, additional studies are needed in order to manage the predisposing factors.

References

1. **Belay, T.K., Dagnachew, B.S., Kowalski, Z.M., Ådnøy, T.,** An attempt at predicting blood β-hydroxybutyrate from Fourier-transform mid-infrared spectra of milk using multivariate mixed models in Polish dairy cattle, Journal of Dairy Science, 2017, 100, 8, 6312-6326.
2. **Benedet, A., Manuelian, C.L., Zidi, A., Penasa, M., De Marchi, M.,** Invited review: β-hydroxybutyrate concentration in blood and milk and its associations with cow performance, Cambridge Journals, 2019, 13, 8, 1676-1689.
3. **Berge, A.C., Vertenten, G.,** A field study to determine the prevalence, dairy herd management systems, and fresh cow clinical conditions associated with ketosis in western European dairy herds, Journal Dairy Science, 2014, 97, 4, 2145-2154.
4. **Chapinal, N., Carson, M.E., LeBlanc, S.J., Leslie, K.E., Godden, S., Capel, M., Santos, J.E.P., Overton, M.W., Duffield T.F.,** The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance, Journal Dairy Science, 2012, 95, 1301-1309.
5. **Chandler, T.L., Pralle, R.S., Dórea, J.R.R., Poock, S.E., Oetzel, G.R., Fourdraine, R.H., White, H.M.,** Predicting hyperketonemia by logistic and linear regression using test-day milk and performance variables in early-lactation Holstein and Jersey cows, Journal of Dairy Science, 2018, 101, 3, 2476-2491.
6. **Duffield, T.F., Lissemore, K.D., McBride, B.W., Leslie, K.E.,** Impact of hyperketonemia in early lactation dairy cows on health and production, Journal of Dairy Science, 2009, 92, 2, 571-580
7. **Grummer, R.R.,** Impact of changes in organic nutrient metabolism on feeding the transition dairy cow, Journal of animal Science, 1995, 73, 9, 2820-33.
8. **Kayano, M., Kataoka, T.,** Screening for ketosis using multiple logistic regression based on milk yield and composition, Journal of Veterinary Medical Science, 2015 77, 11, 1473-1478.
9. **Koeck, A., Jamrozik, J., Schenkel, F.S., Moore, R.K., Lefebvre, D.M., Kelton, D.F., Miglior, F.,** Genetic analysis of milk β-hydroxybutyrate and its association with fat-to-protein ratio, body condition score, clinical ketosis, and displaced abomasum in early first lactation of Canadian Holsteins, Journal of Dairy Science, 2014, 97, 11, 7286-7292.
10. **LeBlanc, S.J., Leslie K., Duffield, T.,** Metabolic predictors of displaced abomasum in dairy cattle, Journal of Dairy Science, 2005, 88, 1, 159-170.
11. **Lee, S.H., Cho, K.H., Park, M.N., Choi, T.J., Kim, S.D., Do, CH.,** Genetic parameters of milk β-hydroxybutyric acid and acetone and their genetic association with milk production traits of Holstein cattle, Asian-Australasian, Journal of Animal Sciences, 2016, 29, 11, 1530-1540.

12. **McArt, J.A.A., Nydam, D.V., Oetzel G.R.,** Epidemiology of subclinical ketosis in early lactation dairy cattle, Journal of Animal Sciences, 2012, 95, 9, 5056-5066.
13. **Rathbun, F.M., Pralle, R.S., Bertics, S.J., Armentano, L.E., Cho, K., Do, C., Weigel, K.A., White, H.M.,** Relationships between body condition score change, prior mid-lactation phenotypic residual feed intake, and hyperketonemia onset in transition dairy cows, Journal of Animal Sciences, 2017, 100, 5, 3685-3696.
14. **Ruoff, J., Borchardt, S., Heuwieser W.,** Short communication: Associations between blood glucose concentration, onset of hyperketonemia, and milk production in early lactation dairy cows, Journal of Animal Sciences, 2017, 100, 7, 5462-5467.
15. **Rodriguez, Z., Shepley, E., Endres, M.I., Cramer, G., Caixeta., L.S.,** Assessment of milk yield and composition, early reproductive performance, and herd removal in multiparous dairy cattle based on the week of diagnosis of hyperketonemia in early lactation, Journal of Dairy Science, 2022, 105, 5, 4410-4420.
16. **Santschi, D.E., Lacroix, R., Durocher, J., Duplessis, M., Moore, R.K., Lefebvre, D.M.,** Prevalence of elevated milk þ-hydroxybutyrate concentrations in Holstein cows measured by Fourier-transform infrared analysis in Dairy Herd mprovement milk samples and association with milk yield and components Lefebvre Valacta, boul, Des Anciens-Combattants, Ste-Anne-de-Bellevue, Journal of Dairy Science, 2016, 99, 11, 9263-9270.
17. **Seifi, S.J., LeBlanc, K.E., Leslie, T.F.,** Duffield Metabolic predictors of post-partum disease and culling risk in dairy cattle, Veterinary Journal, 2011, 188, 2, 216-220.
18. **Silvijo, V., Dražen, Đ., Hrvoje, V., Damjan, G., Ivan, F., Branimira, Š., Przemyslaw, S., Samardžij, M.,** Risk factors and prevalence of subclinical ketosis in dairy cows in Croatia, Veterinarski Arhiv, 2017, 87, 1, 13-24.
19. **Suthar, V.S., Canelas-Raposo, J., Deniz, A., Heuwieser, W.,** Prevalence of subclinical ketosis and relationships with postpar- tum diseases in European dairy cows, Journal of Dairy Science, 2013, 96, 5, 2925-38.
20. **Van der Drift, S.G.A., Jorritsma, R., Schonewille, J.T., Knijn, H.M., Stegeman, J.A.,** Routine detection of hyperketonemia in dairy cows using Fourier transform infrared spectroscopy analysis of β-hydroxybutyrate and acetone in milk in combination with test-day information, Journal of Dairy Science, 2012, 95, 9, 4886-4898.
21. **Vanholder, T., Papen, J., Bemers, R., Vertenten, G., Berge, A.C.B.,** Risk factors for subclinical and clinical ketosis and association with production parameters in dairy cows in the Netherlands, Journal of Dairy Science, 2015, 98, 2, 880-888.

22. **Walsh, R.B., Walton, J., Kelton, D., LeBlanc, S., Leslie, K., Duffield, T.,** The effect of subclinical ketosis in early lactation on reproductive performance of postpartum dairy cows, Journal of Dairy Science, 2007, 90, 6, 2788-2796.

ACTIVITY BUDGETS OF *PANTHERA LEO* IN TÎRGU MUREȘ ZOO, ROMANIA

GRIGOREANU A., GAȘPAR C., LĂZĂRESCU C., COJOCARU R., ȚIBRU I.

Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645,
Calea Aradului No. 119, Timisoara, Romania
E-mail: alexandra.grigoreanu@gmail.com

Summary

The present study investigated diurnal or daily activity patterns of a pair of captive lions (Panthera leo) in Tîrgu Mureș Zoo, Mureș County, Romania comparing to the normal activity of the free ranging lion and other captive lions. Behavioral observations of 147 hours in 21-days observation were analyzed descriptively for common Felidae behaviors identified using a standardized Felidae ethogram. The methodology used was focal animal sampling with continuous sampling of data record or 5-minute sampling periods. We reported that the captive lion allocated the most time budget in inactive behavior or sleeping with the percentage of 67% in the case of the male lion and 65% was the percentage for the lioness. The dominant sleeping behavior may be considered normal to captive Felidae in many zoos. In this case we wanted to see the impact of the habitat and the changes that occur in the daily activity schedule. By knowing the natural activity pattern of an lion and comparing it with the on site pattern we can calculate the impact of an artificial habitat, measuring the wealfare, risks and percentage of a stereotipycal pattern chances of occurrence.
Keywords: ethogram, Panthera leo, time budget.

The ethological study is the first step towards understanding what the wild animal needs in order to be able to live in an artificial environment, more or less close to that existing in the wild (3, 11). So if it can be provided with those minimum safety and area criteria that lead to minimal well-being (8), it is possible to avoid the changes in behaviour and not only that may occur in the context of captivity (3, 10).

Research also shows that the use of indoor space is changing (2). For example, it has been observed that animals kept in enclosures with long escape distances experience less stress than animals kept in small enclosures with short escape distances (6). Behavioral studies are the most common form of non-invasive research to assess the well-being of captive animals (10, 16).

The success of zoo management as ex-situ conservation site for Felidae depends on the animal welfare of captive animals (1). Huge investment on captive Felidae must be supported by the welfare data which can be retrieved from its daily activity pattern or behaviors (2, 14). It has long been reported that big cats may show dominant inactive behavior or frequent stereotypic behavior (4). By comparing the natural activity (4, 5) and other studies we can link the emergence of an pattern with its cause (9, 12) and in this case the changes that can be linked with the artficial enviroment being different from the natural habitat (1, 15).

Materials and methods

The study was conducted at Tîrgu Mureș Zoo, Mureș County, Romania. The two subjects were a 7 year old male African lion named Simon and a 13 year old female African lion named Jesi, both being housed in a 28x28m exterior cage with a 4x4m interior housing.

Behavioral data collection

The study accumulated a total of 8820 minutes (147 hours) which is the time allocated to this study. These audio-video recordings were made so that there is no probability that during the notation (observation) certain behaviors that in the first stage are hardly visible are not omitted. During the study, it was tried to ensure that every day the time allotted for the observation to end in the same time interval except for the first and last day.

An ethogram was used to facilitate a 3-day preliminary assessment of total behaviors. Categorization of lion behaviors are Solitary(Grooming, yawning), Social,Activity(Stretching, Running, Walking, Jumping, Roaring, Urination, Defecation) Rest, Search and Feeding (6, 7). Methodology used was focal animal sampling with continuous sampling of data record or 5-minute sampling periods.

Data analysis

The frequencies of behaviors were combined to form aggregate categories of ‘Active’, ‘Inactive’, and ‘Stereotipy’, the last category being a category that was noted in other studies and it is a key factor in compiling the results. Data were analyzed using Microsoft Excel 2010 in the expression of mean.

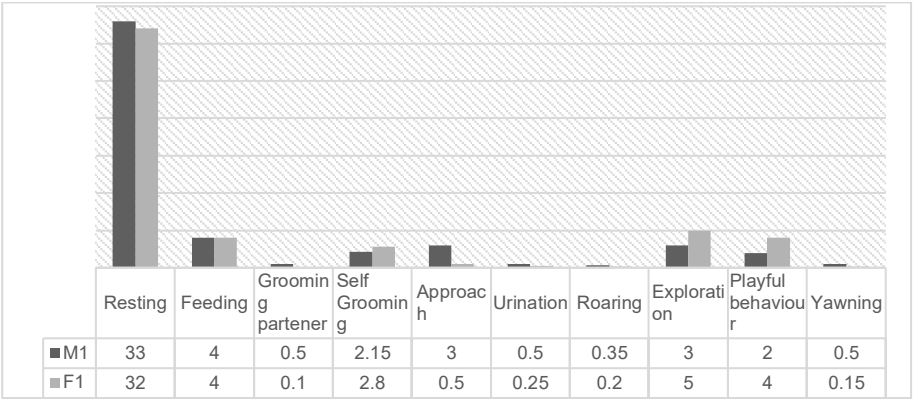
Results and discussions

For the comparing we started from the statement of Schaller that lions may rest up to 20-21 hours per day (9), on average, completed with the statement of Haas that observed a typical pattern of lions in zoos that can sleep 10-15 hours (2).

Of the 49 hours allocated to completing the etogram, over the course of seven days, there were about 33 hours of rest. This behavioral type is specific to the lion, since it, in terms of activity, is a nocturnal animal, and in the wild it spends most of its active life, hunting (4, 13). That is why in captivity, by the absence of prey, the activity itself is only in the morning. The remaining 16 hours of study out of a total of 49 were allocated to different types of behavior, of which the individual behaviors were much more present than the social ones.

The male was found to be the initiator of congeneral care behaviors five times in the first week, but also the one that initiates the approchement between the two lions. On the other hand, the female is more solitary, accepting care for a short period of time, of about 30 seconds/behavior, ending with its withdrawal at a distance of more than 2m from the male. The time that the male allocates, out of the 15 hours, for the care of the partner was 30 minutes, the own care behavior was two hours and 30 minutes, the behavior of closeness was fractionated, over a whole day,

several times, totaling about three hours. On the days when they are given the food, M1 spends four hours searching for their own food and feeding, and after each feeding they mark the territory with urine, the time allotted for marking being 15 seconds. Most of the time, it was found that the sonorous expression was due to the closeness of the other lions. It was found that in the first week, the sound activity reached 35 minutes (Fig. 1).



	Resting	Feeding	Grooming partener	Self Grooming	Approach	Urination	Roaring	Exploration	Playful behaviour	Yawning
M1	33	4	0.5	2.15	3	0.5	0.35	3	2	0.5
F1	32	4	0.1	2.8	0.5	0.25	0.2	5	4	0.15

Fig. 1. Time budget in hours

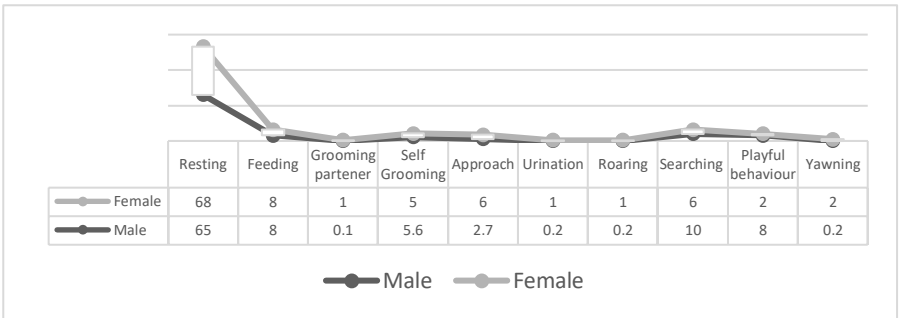
F1, compared to M1, expresses playful behavior much more frequently, observing an affinity towards objects in the habitat. The social behavior expressed by her towards M1 is much rarer and if engagements occur in these behaviors of M1, it ends with the withdrawal and interruption of the behavior, an action performed by F1. F1 is not considered to be the cause of the sound expression, as it is the receiver for the M1, but it can be seen that F1 is much more curious, often expressing the curiosity behavior towards visitors, congeners, but also caregivers.

The time allotted to sleep, as far as F1 is concerned, compared to M1 (34 hours) is reduced to 32 the two hours being recorded as the time allocated to playful behavior. The remaining 15 hours, statistically are divided differently from M1, so we can conclude that there are differences between congeners even if they are subject to the same environmental factors and are placed in the same habitat. Thus, the data collected can be considered as a way of observing the specificity of the individual, a phenomenon often observed in other animals (11).

Regarding the feeding behavior F1 allocates about four hours and 25 minutes to this behavior, fractionating the behavior into several stages, as follows: the search for food is carried out in several halves / day for two minutes, sniffing and analyzing the food 10 seconds / approaching, taking food from the ground takes 2.5 seconds / opening the mouth, and the actual feeding is carried out for five minutes. F1 engages in this behavior, several times a day, at a relatively constant interval, so

it is noticed that on the days of feeding, the lioness feeds at least twice every 45 minutes.

From the observations made, we can conclude a percentage difference between the two sexes, but also by the fact that, although the male is more inactive in terms of the percentage that allocates it to the activities itself, it seems that it has a series of behaviors performed that the female does little or not at all, so it can be considered that there is a sex specificity regarding the behavior of the two individuals (Fig. 2).



	Resting	Feeding	Grooming partener	Self Grooming	Approach	Urination	Roaring	Searching	Playful behaviour	Yawning
Female	68	8	1	5	6	1	1	6	2	2
Male	65	8	0.1	5.6	2.7	0.2	0.2	10	8	0.2

Fig. 2 Percentage of activities during the study

As a percentage, the female expresses more playful behavior than her congener, but no sound emitted by her is recorded. The lower percentage allocated to resting behavior, is due to the role of the female as more active in the relationship between the two individuals.

At the end of the study the time budget separation model exposes certain information from which we can formulate our own assumptions thus:

Assumption 1

The social relationship is influenced by the daily activity of the two lions. The male being inactive in terms of daily activities, demonstrated by the fact that he allocates a lot of time to rest, the occasions when the two lions can have social interactions are greatly diminished, compared to what can be observed in the natural environment. We can reinforce this hypothesis by motivating the fact that in nature in order to survive, the sociality of lions is dominated by the desire to obtain food, which in captivity is not observed.

Assumption 2

The social relationship is influenced by the gender and age of the two individuals. If we take into account the physiology of lions and the way of relating in nature we can support this hypothesis by the statement that the male-female relationship is of mutual acceptance, not of social strengthening, and due to the fact that the female is in anestrous, she is not interested in the male and vice versa.

Assumption 3

The social hierarchy can be outlined as the dominant lioness and the dominated male, reinforcing this statement corroborating all the records in which the female refuses the social intentions of the male and he resigns himself.

Conclusions

We can conclude that, in relation to the time budgets, there is a discrepancy in the daily activity of the lions, which has a significant impact in the social relationship of habituation of the two lions taken into account. The time budget separation model exposes certain information from wich we can incorporate our assumptions as being a individual characteristic and thus having different activity during a day.

References

1. **Adam, F.,** Feline behaviour and welfare, Ed. Cambrige, 2012.
2. **Andrew, G., Weiss, M.,** Personality in felids: a review, Journal of Applied Animal Behaviour Science, 2013, 144, 1-2, 1-13.
3. **Anthony, R.P.,** Studying Captive Animals: A Workbook of Methods in Behaviour, Welfare and Ecology, Ed. John Wiley & Sons, Elsevier, 2015.
4. **Bauer, H., Packer, C., Funston, P.F., Henschel, P., Nowell, K.,** Panthera leo, The IUCN Red List of Threatened Species, 2016.
5. **Chosy, J., Wilson, M., Santymire, R.,** Behavioural and physiological responses in felids to exhibit construction, Journal of Zoo Biology, 2014, 33, 4, 267-274.
6. **Kleiman, D.G.,** Wild Mammals in captivity Principles and Techniques for Zoo Management, Second Edition, Ed. University of Chicago Press, 2010.
7. **Loarie, S.R., Tambling, C.J., Asner, G.P.,** Lion hunting behaviour and vegetation structure in an African savanna, Journal Animal Behaviour, 2013, 85, 700-900.
8. **Lyniuk, I.L.,** Wild Cats in Captivity: On Improving Captive Environments with Regard to Biology and Behaviour-Thesis, 2011.
9. **Michaell, S., Sullivan, L., Fazio, S.,** A standardized ethogram for the felidae: a tool for behavioral researchers, Journal of Applied Animal Behaviour Science, 2015, 173, 10-25.
10. **Packer, C., Pusey, A.E., Ebley, L.E.,** Egalitarianism in female African lions, Science, 2010, 293, 690-693.
11. **Pastorino, G.,** Role of Personality in Behavioral Responses to New Environments in Captive Asiatic Lions, Veterinary Medicine International, 2017, 1-17.
12. **Putman, S.B., Brown, J.L., Franklin, A.D., Schneider, E.C., Boisseau, N.P., Asa, C.S., Pukazhenthi**, **B.S.,** Characterization of Ovarian Steroid Patterns in

Female African Lions (Panthera leo), and the Effects of Contraception on Reproductive Function, PLoS One, 2015, 10, 135-377.
13. **Schmid, B.,** Reduced temporal partitioning among Africa's large carnivores, Journal of Ecology, 2012, 2580-2598.
14. **Swaisgood, R.R., Schulte, B.A.,** Applying knowledge of mammalian social organization, mating systems, and communication to management, Ed. Univ. Of Chicago Press, Chicago, 2010.
15. **VanderWaal, K.L., Mosser, A., Packer, C.,** Optimal group size, dispersal decisions and post dispersal relationships in female African lions, Journal of Animal Behaviour, 2009, 949-954.
16. **Weiss, A., Murayama, I., Kawamura, S.,** From Genes to Animal Behavior: Social Structures, Personalities, Communication by Color, Ed. Springer, Tokyo, 2011.

EPIDEMIOLOGICAL EVALUATION AND CLINICAL FEATURES OF FELINE PKD

HUŞTEA (DECEI) L., CRISTIAN A., CODREANU M.

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
Faculty of Veterinary Medicine, 011464,
Mărăşti Blvd No. 59, District 1, Bucharest, Romania
E-mail: decei.liliana@yahoo.com

Summary

Polycystic kidney disease is characterized by a progressive, stage-type evolution, being dominated by clinical manifestations specific to chronic kidney disease. The study was conducted during 2014-2018, within the Clinic of the Faculty of Veterinary Medicine Bucharest and in the private veterinary practices Canivet and Vet Medical Consulting SRL, on a number of 21 cats, presented at the clinic with symptoms suggestive of chronic kidney disease, who were subjected to specific stages of clinical examination. The results obtained indicate a high prevalence among the Persian race (64%) and a significant incidence in the age group of 6-10 years (56%). The neuromuscular repercussions in nitrogen retention syndrome are mainly represented by a depressive syndrome and a precomatous condition detected with a high intensity in 11 cats (52.38%). In terms of body weight, 13 cats underwent severe changes with a considerable loss of body weight. As a result of the interpretation and assessment of body temperature in patients with CKD, hypothermia was recorded in 66.67% of individuals (n = 14) and the presence of a body temperature within physiological limits in relation to species and age in 33.33% of patients (n = 7). The analysis of the anamnesis and clinical signs identifies the presence of severe anorexia in 14 patients (66.67%) and moderate in a percentage of 19.05% (n = 4), the presence of vomiting, consequence of increased serum uremia and uremic gastritis, at 7 patients (33.33%) and diarrhea syndrome in 4 cats. Polyuria-polydipsia syndrome was a specific symptom of orientation in the diagnosis of chronic kidney disease, being identified in a number of 16 patients (76.19%). The symptomatology in polycystic kidney disease has the character of orientation towards the functional diagnosis, the clinical aspects relevant for the chronic renal insufficiency, followed by its confirmation by paraclinical methods.

Keywords: polycystic kidney disease, felines, chronic kidney disease.

Polycystic kidney disease is characterized by a progressive, stage-type evolution, being dominated by clinical manifestations specific to chronic kidney disease (10, 18, 19, 20).

The symptomatology is generated by the development of these cystic formations at parenchymal level, producing progressively, by compression and substitution, the reduction of the functional capacity of the kidneys. Subsequently, the consequences of nitrogen retention are added. Chronic kidney disease usually takes the form of uremic (azotemic) syndrome expressed by progressive weight loss with hypothermia (rectal temperature can reach 34˚C), digestive symptoms, cortical

depression or uremic coma phase, and polyuria-polydipsia syndrome (3, 4, 8, 15, 17).

Materials and methods

This research includes 21 cats, presented at the clinic with symptoms suggestive of chronic kidney disease, who underwent specific stages of clinical examination, followed by paraclinical investigations to detect changes in biochemical and hematological parameters and ultrasound evaluation for the diagnosis of polycystic kidney disease.

The group of patients included in the study the Persian breed numbering 14 patients, the British Shorthair breed with 2 cats, 2 cats of European breed, Exotic Shorthair - 2 cats, and the Scottish Fold 1 feline, aged 0-15 years , female (n = 14) and male (n = 7) (Table 1).

Table 1

Structure of the group of patients symptomatic with CKD

NUMBER OF GROUP PATIENTS (n=21)					
BREED		**AGE**			**SEX**
		1-5 years	**6-10 years**	**11-15 years**	**M/F**
Persian	***(n=14)***	***(n=6)***	***(n=14)***	***(n=1)***	**F=7 M=14**
British Shorthair	***(n=2)***				
Exotic Shorthair	***(n=2)***				
Scottish Fold	***(n=1)***				
European	***(n=2)***				

Results and discussions

Following the analysis of the prevalence of polycystic kidney disease in cats, it is found that among the Persian cat population were diagnosed 14 individuals with a percentage of 64.00% of the study group, 2 British Shorthair patients (8.00%), 2 cats Exotic Shorthair with a percentage of 8.00% (n = 2), 2 European cats (16.00%) and a Scotish Fold cat (4.00%) (Fig. 1) in accordance with the published articles (1).

The affected age group introduced in the study is directly proportional and closely related to the establishment and functional expression of chronic kidney failure, generated by the presence of specific cysts.Thus, in the research, cats with PKD diagnosed were included in the following age ranges - 1-5 years (n = 6), cases in which the owners were informed of the importance of periodic evaluation or clinical manifestations were sufficiently expressed, 6- 10 to a number of 14 animals with a percentage of 66.67%, being the category most prone to the development and

expression both functional and physical of the pathogenetic consequences of the disease and category 11-15 in 1 case (4.76%).

The results obtained in the prevalence of PKD by age were illustrated graphically in the Fig. 2.

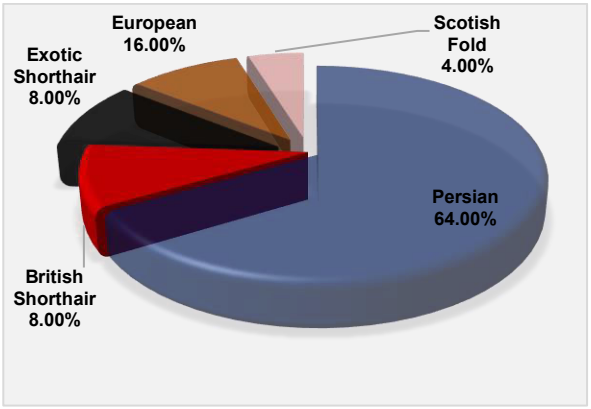


Fig. 1. Distribution of race prevalence in individuals with PKD

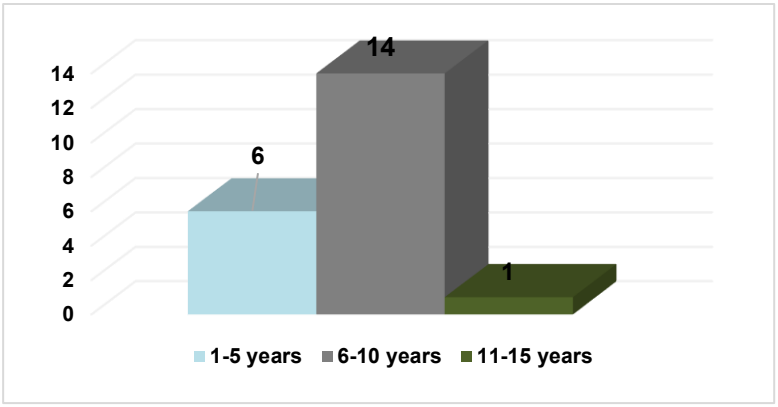


Fig. 2. Distribution of age prevalence in individuals with PKD

Previous research on cats with PKD shows mixed results in terms of sex incidences in evaluated cats, thus Barthez et al. (1) in 2003, and Domanjko-Petrič et al. (11) in 2008 suggests that there is no predilection for sex, but in the study conducted in Thailand there is an increased incidence of females, which is also observed in this research (Fig. 3), where the proportion of affected females is 66.67% (n = 14) compared to males where the percentage is 33.33% (n = 7) (2, 11, 16).

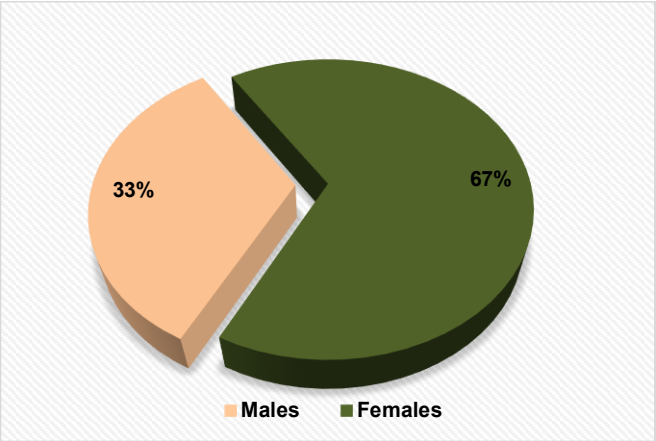


Fig. 3. Distribution of sex prevalence in individuals with PKD

The symptoms in polycystic kidney disease are oriented towards functional diagnosis, through the clinical aspects relevant for chronic kidney disease, represented by the general signs observed in the study, weight loss and body temperature, gastrointestinal signs expressed mainly by anorexia / loss of appetite, oral disorders determined of uremic syndrome, gingival ulcers, halitosis or somatitis, vomiting and hematemesis and intestinal disorders expressed by diarrhea (4, 13).

The degree of weight loss suggests the period in which the body globally feels biochemical and morphological disorders with the consequent reduction of body weight, being mainly caused by anorexia or loss of appetite, repeated vomiting and frequent diarrhea (9).

The patients included in the study were evaluated, in terms of body weight, being obtained the category of animals with a slight weight loss with a body weight between 5-6 kg (n = 1), cats that recorded a moderate weight loss (n = 7) having a weight between 4-5 kg and animals that expressed a severe reduction in body weight (n = 13) with a percentage of 61.90% with a body weight between 1.9-3 kg (Fig. 4).

Body temperature marks an unfavorable prognosis for the patient, being a viable indicator in order to assess the degree of serum uremia responsible for the common hypothermia (6).

Following the interpretation and assessment of body temperature in patients with CKD, a low internal temperature was recorded in 66.67% of individuals (n = 14) and the presence of a body temperature within physiological limits in relation to species and age at 33.33% of patients (n = 7) (Fig. 5).

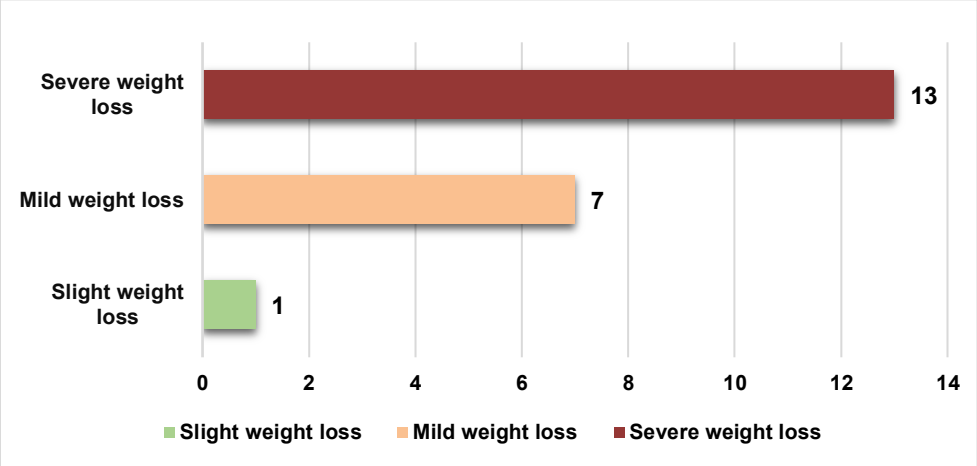


Fig. 4. Recorded body weight in patients diagnosed with CKD

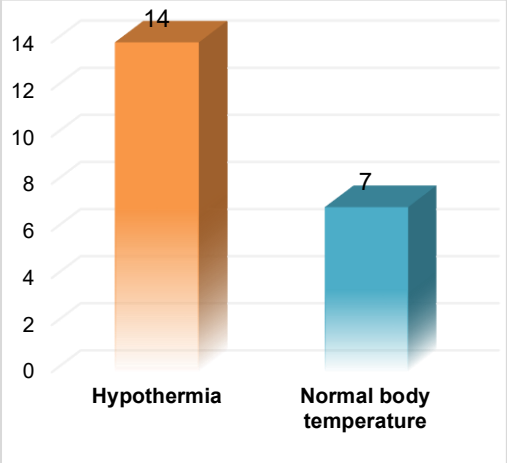


Fig. 5. Graphical systematization of body temperature in patients with CKD

Gastrointestinal disorders generated by uremic syndrome have value in guiding the diagnosis of chronic kidney disease by associating clinical elements that taken individually have no pathognomonic value, but in the unitary clinical context analyzed indicates a diagnosis of suspicion (7, 12).

In the research on the investigated animals, suggestive clinical gastrointestinal elements were represented by the presence or absence of halitosis,

anorexia / loss of appetite, food vomiting or hematemesis, uremic stomatitis due to mouth ulcers and diarrhea syndrome (10, 12).

Halitosis or halena was identified as severe in 5 patients and moderate in 5 individuals.

Modified appetite expressed as anorexia, where food refusal was total or loss of appetite, where animals had a reduced appetite due to gastrointestinal and metabolic disorders, being assessed and evaluated severely in 14 patients (66.67%) and moderate in a percentage of 19.05% (n = 4).

Ulcerative stomatitis, caused by the elimination of serum urea through the gingival mucosa, was diagnosed on the basis of mouth ulcers and inflammation of the gingival mucosa in 2 individuals (9.52).

The analysis of the anamnesis and clinical signs identifies the presence of vomiting, a consequence of increased serum uremia and uremic gastritis, in 7 patients (33.33%), with ammoniacal odor, and diarrhea was detected in 4 patients (19.05%) expressed as acute diarrhea, from the small intestine in aqueous form or with undigested food residues (Fig. 6).

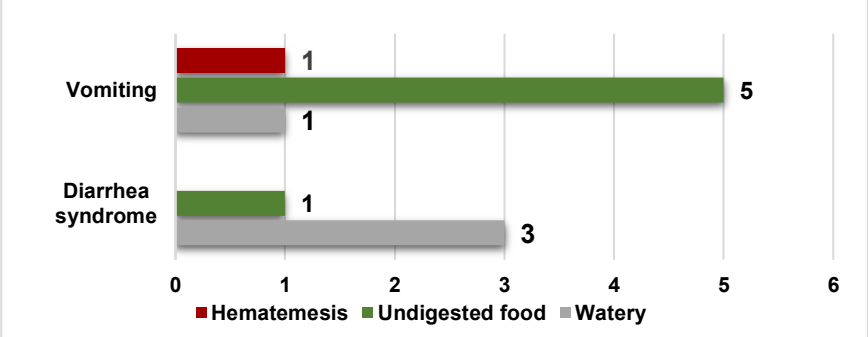


Fig. 6. Systematization of the main digestive manifestations in CKD

The neuromuscular repercussions in nitrogen retention syndrome, observed in our research, are mainly represented by a high-intensity depressive syndrome detected in 11 cats, and a precomatous state expressed by reduced reflexes and deeply affected physiological constants, obtaining a percentage of 52,38%, average intensity in 3 cats (14.29%), low intensity in a percentage of 23.81% (n = 5) and absent in 2 patients (Fig. 7).

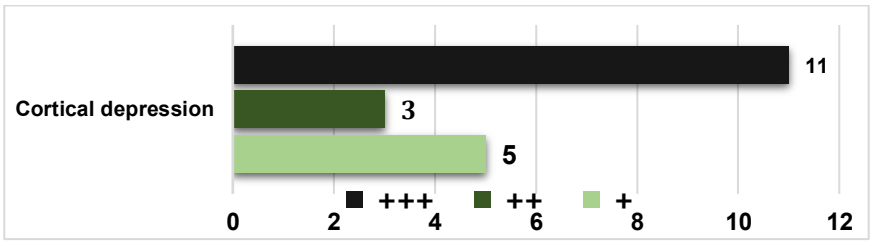


Fig. 7. Degrees of intensity of depression syndrome in individuals diagnosed with CKD

Polyuria-polydipsia syndrome, generated by a decrease in the number of functional nephrons with an increase in tubular flow in the remaining nephrons and the appearance of a diluted diuresis (5), was a specific symptom of guidance in the diagnosis of chronic kidney disease, being identified in 16 patients (76.19%) and absent in 5 individuals (Fig. 8).

The intensity with which the polyuria-polypsipsia syndrome was expressed was quantified with low intensity, being captured by the owners in 2 cats (9.52%), average intensity recognized by both the owner and by the veterinarian at a percentage of 14.29% and high intensity detected in cats at a percentage of 52.38% (n = 11) in individuals.

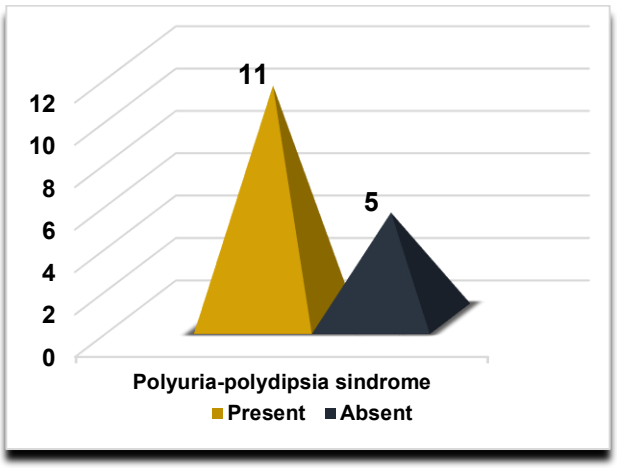


Fig. 8. Presence of polyuria-polydipsia syndrome in individuals diagnosed with CKD

Conclusions

The symptomatology in polycystic kidney disease has the character of orientation towards the functional diagnosis, followed by its confirmation by paraclinical methods.

The clinical coordinates indexed in the evaluation indicated a symptomatic polymorphism, dominated by specific clinical aspects that by group appreciation, aims to guide the diagnosis of suspicion, of chronic kidney disease determined by polycystic kidney disease.

References

1. **Bartez, P.Y., Rivier, P., Begon, D.,** Prevalence of polycystic kidney disease in Persian and Persian related cats in France, Journal of Feline Medicine and Surgery, 2003, 5, 6, 345-347.
2. **Bartges, J., Polzin, D.J.,** Nephrology and Urology of Small Animals, Ed. Wiley Blackwell, Tennessee, SUA, 2011.
3. **Bartges, J.W.,** Chronic kidney disease in dogs and cats, Veterinary Clinics of North America: Small Animal Practice, 2012, 42, 4, 669-692.
4. **Biller, D.S.,** Polycystic kidney disease, Consultations in Feline Internal Medicine Vol. 2, J.R. August Elsevier, St Louis, 1994.
5. **Biller, D.S., Dibartola, S.P., Eaton, K.A., Pflueger, S.P., Wellman, M.L., Radin, M.J.,** Inheritance of Polycystic Kideny Disease in Persian Cats, Journal of Heredity, 1996, 87, 1, 1-5.
6. **Biller, D.S., Chew, D.J., Dibartola, S.P.,** Polycystic kidney disease in a family of Persian cats, Journal of the American Veterinary Medicine Association, 1990, 196, 8, 1288-1290.
7. **Biller, D.S.,** Eaton KA, DiBartola S.P, Radin M.J, Wellman M.L. Autosomal dominant polycystic kidney disease in Persian and Persian-cross cats, Veterinary Pathology Journal,1997, 34, 2, 117-26.
8. **Codreanu, M.D., Gerwing, M.,** Aspecte clinice si paraclinice in boala polichistică renală la pisică, Revista Română de Medicină Veterinară, 2003, 13, 3-4, 300.
9. **Codreanu, M.D.,** Clinical, hematological, biochemical and ultrasonographic changes kidney disease (PKD) in cat, The Sixth International Symposium in animal clinical pathology and therapy, Clinica Veterinaria, 2004, 18-21.
10. **Codreanu, M.,** Patologia medicală a animalelor domestice, Bolile aparatului urinar, Ed. Ex Terra Aurum, București, 2020.
11. **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.
12. **Ferreira, G.S., Galvão, A.L.B., Socha, J.M.,** Polycystic kidney disease in cats: a literature review, Anales de Veterinaria de Murcia, 2010, 26, 23-31.

13. **Pei, Y.,** Diagnostic approach in autosomal dominant polycystic kidney disease, Clinical Journal of the American Society of Nephrology, 2006, 1, 5, 1108-14.
14. **Preda (Constantinescu), V., Popa (Cristian), A.M., Codreanu, M.,** Dominante clinico patogenetice în nefropatiile polichistice la animalele de companie, Catlife, 2017, 12, 31-35.
15. **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, 81-82.
16. **Supamanee, N., Sattasathuchana, P., Nilkumhang, P., Teerapan, W., Wajjawalku, W., Thengchaisri, N.,** Survey of feline polycystic kidney disease in 102 Persian and Persian-related cats by ultrasonography, Kasetsart Veterinarians, 2010, 20, 2, 77-84.
17. **Syme, H.M.,** Polyuria and polydipsia, BSAVA Manual of Canine and Feline Nephrology and Urology, Second edition, J Elliott and GF Grauer, SUA, 2007.
18. **Welling, L.W., Grantham, J.J.,** Cystic and developmental diseases of the kidney, Ed. Philadelphia, W.B. Saunders,SUA, 1991.
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. **Yoshihiko, Y.U., Shumway, K.L., Matheson, J.S., Edwards, M.E., Kline, T.L., Lyons, L.A.,** Kidney and cystic volume imaging for disease presentation and progression in the cat autosomal dominant polycystic kidney disease large animal model, BMC Nephrology, 2019, 20, 1, 3-11.

DIVERSITY OF TICK SPECIES SAMPLED FROM RECREATIONAL AREAS IN ARAD MUNICIPALITY

IMRE M.[1], MORARIU S.[1], OPRESCU I.[1], IMRE K.[1], FLOREA T.[1], LUCA I.[1], TRUŢ P.A.[2], NAGY I.[1], DĂRĂBUŞ G.[1]

[1]Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645, Calea Aradului, No. 119, Timisoara, Romania
[2]Mcm-Sanitfarmvet, Deva
E-mail: mirela.imre@usab-tm.ro

Summary

Recreational areas are important in the urban habitat, but may act as favoring factors in the spreading of parasites such as ticks if the vegetation is abundant and the hosts are present. The study was undertaken to provide data on tick diversity from several recreational areas of Arad municipality and to evaluate the possible risk for the public health and companion animals. The methodology of the present study was based on 362 ticks collected using the dragging method from 5 locations from Arad Municipality and its surrounding areas, including public parks and forest areas. The ticks were identified using a stereomicroscope and the SEM method. The following species and genera were found*: Ixodes ricinus*, *Dermacentor reticulatus*, *Haemaphysalis* spp. The number of collected ticks varied among the studied areas from 106 (highest value) in Area 5 – park to 20 (lowest value) in Area 1 – forest zone. *Ixodes ricinus* and *Dermacentor reticulatus* were mainly found in park areas, while *I. ricinus* was better represented in forest areas. The identification of a high number of *I. ricinus* nymphs in forest areas draws attention on the possibility of transmission of *Borellia* spp. in humans. In addition, they can also harbor several other tick-borne pathogens. *Dermacentor reticulatus,* found mostly in parks frequented by dogs, highlighted the possibility of transmission of *Babesia* spp. in dogs. Therefore, people visiting recreational areas like public parks or woodlands together with their companion animals should be aware that may be at risk of coming into contact with these parasites and the pathogens transmitted by them.

Keywords: ticks, forest, park, dragging.

Ixodidae ticks or hard ticks (Arthropoda phylum) are considered one of the most important vector groups for spreading important pathogens, including bacteria, helminths, protozoa or viruses (6, 7, 8, 9, 12, 19, 20).

Canine babesiosis is transmitted by *Dermacentor reticulatus* (*Babesia canis*), *Rhipicephalus sanguineus* (*B. canis, B. vogeli* and *B. gibsoni*) and *Haemaphysalis* spp. (*B. rossi and B. gibsoni*) and canine erlichiosis and anaplasmosis are transmitted by *Rh. sanguineus* and *Ixodes* spp., all three diseases being important tick-borne conditions. The Lyme disease or borreliosis is transmitted by *Ixodes ricinus* and the "Rocky Mountain spotted fever" by *Dermacentor andersoni* and *D. variabilis*, respectively. Hepatozoonosis, a relatively common canine protozoan infection especially in southern Europe, is transmitted by *Rh. sanguineus*.

The present study was undertaken to determine the diversity of the tick species collected from the recreational areas of Arad Municipality, based on their morphological characteristics, using classical methods and scanning electron microscopy (SEM) technique, in order to assess the risk of transmission of pathogens by these ectoparasites.

Materials and methods

The samples were collected from March to June 2018, using the dragging method described by Sonenshine 1993 (16, 17, 18). Five locations from Arad town and from the surrounding areas were included in the study, as are presented in the Fig. 1. Locations were two forests and three park areas, frequently used as recreational zones by dog owners walking with their pets. The ticks were collected from the harvest cloth using tweezers and stored into separate containers for each location. The samples were transported to the laboratory in containers with 70% ethanol for better preservation. Tick species identification was carried out acccording to specific guidelines from the scientific literature (2, 3, 9).

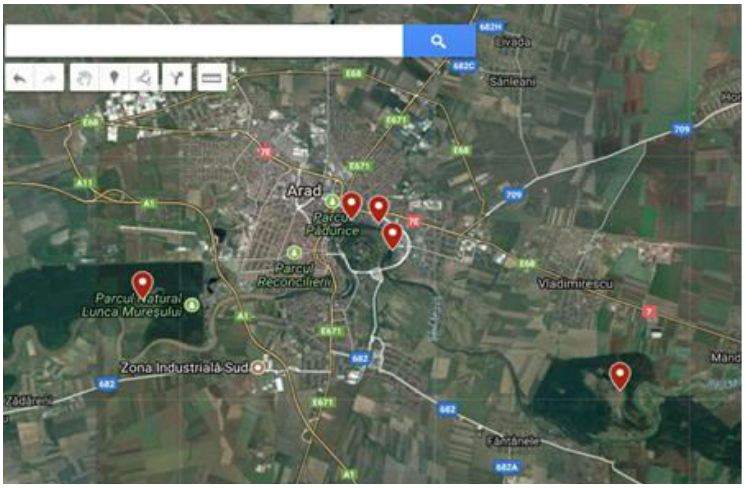


Fig.1. Map of the collection sites (red dots with white circle) for ticks representing the Arad Municipality and its surrounding areas

Results and discussions

A total of 362 ticks, from three parks were collected and identificated at species level. Overall, the light microscopy examination of the 362 environmental origin ticks revealed the presence of the following species and genera: *Ixodes*

ricinus, Dermacentor reticulatus and *Haemaphysalis* spp. The tick distribution according to the five sampled locations are summary presented in Table 1, Fig. 2.

The number of collected ticks varied among the studied areas from 106 (highest value) in Area 5 – park to 20 (lowest value) in Area 1 – forest. *Ixodes ricinus* and *Dermacentor reticulatus* were the most frequently identified in park areas, while *I. ricinus* alone was better represented in forest regions.

Table 1

Distribution of the collected tick species according to their location, number and gender

Location	No. ticks	*Ixodes ricinus*		*Dermacentor reticulatus*		*Haemaphysalis spp.*		Nimphs
		F	M	F	M	F	M	
Collection site 1 -forest	**20**	6	2	-	-	10	-	2
Collection site 2 forest	**72**	18	22	4	-	-	-	28
Collection site 3 – park	**88**	22	38	4	4	20	-	-
Collection site 4 park	**76**	28	32	6	-	6	-	4
Collection site 5 - park	**106**	42	34	6	6	16	-	2

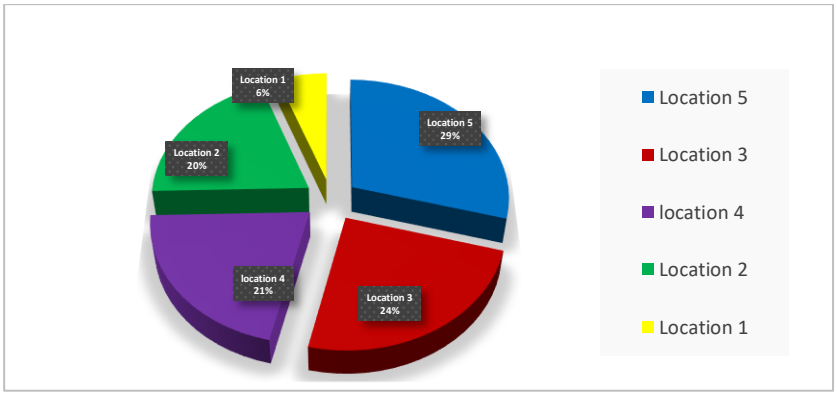


Fig. 2. General distribution of the collected ticks

In the recreational forest areas, the species *Ixodes ricinus* was recorded as dominant, with a large number of nymphs presents in Location 2 -Forest, but also

Haemaphisalis spp., possible due to the presence of grazing livestock (sheep, cattle, goats) in the area (Fig. 3.), has been also recorded.

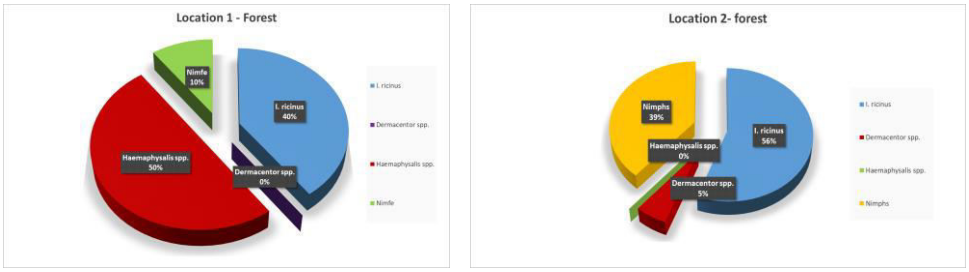


Fig. 3. Graphical representation of tick species collected in the forest areas

In the recreational park areas 3, 4 and 5 the dominant specie was *I. ricinus,* followed by *Haemaphisalis* spp. and *Dermacentor* spp. (Fig. 4).

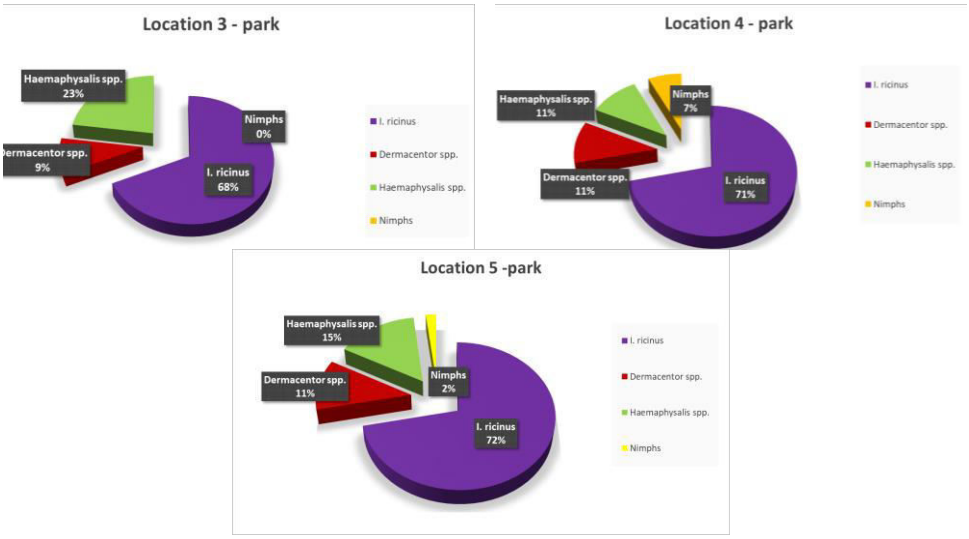


Fig. 4. Graphical representation of tick species collected from the park areas

The available data from the scientific literature pointed out that *I. ricinus* has also been reported as the most common species of tick in dogs from Italy (10), Hungary (4), Great Britain (14), and Germany (1). This species is considered an important reservoir and vector for the transmission of the zoonotic pathogen *Borrelia burgdorferi*. The presence of this pathogen in the Banat area has been previously reported in a study based on immunofluorescence tools in dogs, concluding that the

possibility of it being transmitted to humans through *I. ricinus* being very high (7) In another serological investigation conducted by Hristea et al. (5) the seroprevalence of anti-*Borrelia burgdorferi* antibodies in humans had values of up to 8.7% in blood donors in Maramureş county, and 31.7% in forest workers in Arad County (5).

In a study conducted in Bavaria (Germany) by Schorn et al. (15), it was concluded that the pathogens carried by ticks appear both in forests, as well as in recreational and urban areas (15). This demonstrates the importance of systematic investigation of different habitat types in order to obtain important information about pathogens and ticks. In another screening conducted in Denmark by Klitgaard et al. (11), a total of 509 forest origin nymphs and 504 adult females of *I. ricinus* were molecularly examined. The obtained results demonstrated the carriage status of tick for several important pathogens including *Borellia* spp. *Anaplasma phagocytophilum* and *Rickettsia Helvetica* (11).

Dermacentor reticulatus ticks are the main invertebrate hosts involved in the transmission of canine babesiosis. Considering the large number of cases of babesiosis diagnosed by the veterinarians in the Arad city and surrounding area in the recent years (Imre unpublished results), our hypothesis is that parks of the recreational area can be considered risk factor for *Babesia* infection in dogs. These tick species can also be implicated in the transmission of other pathogens such as *Anaplasma* spp. and *Rickettsia* spp., of which presence has not been previously investigated in the area (7, 12).

In a study in Germany, nine public parks in five Bavarian cities were screened for the presence of ticks. In 2010, in the same study, 5 other different locations were investigated. Collection of samples was carried out monthly (from April to September in 2009 and from May to September in 2010), from vegetation, using the “flagging” method. A total of 9107 ticks were collected in 2009 and 4296 in 2010. All collected ticks belonged to the genus *Ixodes* and *Ixodes ricinus* species. It was concluded that the pathogens carried by ticks occur both in forest as well as in recreational and urban areas. This study demonstrates the importance of systematic investigation of different habitat types in order to obtain important information about ticks and they harbored pathogens (15).

In another study conducted in France, 50 nymphs of the genus *Ixodes ricinus* were collected. These were identified by specific morphological criteria and tested by PCR technique, to detect possible bacteria or protozoa carried. 70% (35/50) of the investigated ticks were positive for at least one microorganism, 26% (9/35) were positive for 2 or more species of microorganisms. A number of human-specific pathogens have been identified, namely: *Borellia burgdoferi*, *Rickettsia helvetica*, *Babesia venatorum*, *Bartonella spp*. (13).

Conclusions

The present study demonstrated that the recreational areas of Arad Municipality are populated by the *Ixodes ricinus*, *Dermacentor reticulatus* and *Haemaphysalis* tick species. *Ixodes ricinus* and *Dermacentor reticulatus* were identified predominantly in parks. In the recreational forest areas, the dominant occurrence of *Ixodes ricinus* was observed.

I. ricinus, identified as the most prevalent tick species in the screened recreational areas of Arad Municipality, can constitute a risk factor for the human population, being recognized as an important reservoir and vector for the zoonotic infectious pathogen *Borrelia burgdorferi*.

The transmission risk for *Babesia* species to dogs in the investigated area was confirmed by the occurrence of its main vector, namely *Dermacentor reticulatus*.

References

1. **Beichel, E., Petney, T.N., Hassler, D., Brückner, M., Maiwald, M.,** Tick infestation patterns and prevalence of *Borrelia burgdorferi* in ticks collected at a veterinary clinic in Germany, Veterinary Parasitology, 1996, 65, 147-155.
2. **Chițimia, L.,** Căpușe Ixodide, Ed. Mirton, Timișoara, 2007.
3. **Estrada-Pena, A., Bouattour, A., Camicas, J.L., Walker, A.R.,** Ticks of domestic animals in the Mediterranean region. A guide of identification of species, Ed. ICTTD, University Zaragoza, Spain, 2004.
4. **Földvári, G., Farkas, R.,** Ixodid tick species attaching to dogs in Hungary, Veterinary Parasitology, 2005, 129, 125-131.
5. **Hristea, A., Hristescu, S., Ciufescu, C., Vasile, A.,** Seroprevalence of *Borrelia burgdorferi* in Romania, European Journal of Epidemiology, 2001, 17, 891-896.
6. **Ilie, M.S., Darabus, G., Imre, M., Imre, K., Hotea, I., Sorescu, I.,** Survey of canine babesiosis in Banat area, Bulletin UASVM Veterinary Medicine, 2010, 67, 125-130.
7. **Imre, M.,** Cercetări privind epidemiologia diagnosticul și controlul babesiozei canine în vestul României, PhD thesis, 2013, Faculty of Veterinary Medicine Timișoara.
8. **Imre, M., Dudu, A., Ilie, M.S., Morariu, S., Imre, K., Dărăbuş, G.,** Molecular survey of *Hepatozoon canis* in red foxes (*Vulpes vulpes*) from Romania, Journal of Parasitology, 2015, 101, 490-491.
9. **Imre, M., Ilie, M.S., Mihali, C.V., Oprescu, I., Morariu, S., Hotea, I., Imre, K., Ilie, A., Palca, M., Dărăbuș, G.,** Prevalence of tick species in dogs using classical methods and SEM, Lucrari Stiintifice - Universitatea de Stiinte Agricole a Banatului Timisoara, Medicina Veterinara, 2012, 45, 131-136.
10. **Iori, A., Gabrielli, S., Calderini, P., Moretti, A., Pietrobelli, M., Tampieri, M., Galuppi, R., Cancrini, G.,** Tick reservoirs for piroplasms in central and northern Italy, Veterinary Parasitology, 2010, 170, 291-296.

11. **Klitgaard, K., Kjær, L.J., Isbrand, A., Hansen, M.F., Bødker, R.,** Multiple infections in questing nymphs and adult female Ixodes ricinus ticks collected in a recreational forest in Denmark, Ticks and Tick-borne Diseases, 2019, 10, 5, 1060-1065.
12. **Morar, D., Dărăbuş, G., Imre, M., Ilie, M.S., Imre, K.,** First record of autochthonous canine ehrlichiosis caused by *Ehrlichia canis* in Romania, Veterinary Clinical Pathology, 2015, 44, 200-204.
13. **Nebbak, A., Dahmana, H., Almeras, L., Raoult, D., Boulanger, N., Jaulhac, B., Mediannikov, O., Parola, P.,** Co-infection of bacteria and protozoan parasites in *Ixodes ricinus* nymphs collected in the Alsace region, France, Ticks and Tick-borne Diseases, 2019, 10, 6,101241.
14. **Ogden, N.H., Cripps, P., Davison, C.C., Owen, G., Parry, J.M., Timms, B.J., Forbes, A.B.,** The ixodid tick species attaching to domestic dogs and cats in Great Britain and Ireland, Medical and Veterinary Entomology, 2000,14, 332-338.
15. **Schorn, S., Pfister, K., Reulen, H., Mahling, M., Silaghi, C.,** Occurrence of *Babesia* spp., *Rickettsia* spp. and *Bartonella* spp. in *Ixodes ricinus* in Bavarian public parks, Germany, Parasites and Vectors, 2011, 4, 135.
16. **Sonenshine, D.E.,** Biology of Ticks, vol. 2, Oxford University Press, NY, 1993.
17. **Springer, Y.,** TOS Protocol and Procedure: Tick and Tick-Borne Pathogen Sampling, 2015.
18. **Tack, W., Madder, M., De Frenne, P., Vanhellemont, M., Gruwez, R., Verheyen, K.,** The effects of sampling method and vegetation type on the estimated abundance of Ixodes ricinus ticks in forests, Experimental and Applied Acarology, 2011, 54, 285-292.
19. **Trotz-Williams, L.A., Trees, A.J.,** Systematic review of the distribution of major vector-bone parasitic in dogs and cats in Europe, Veterinary Record, 2003, 152, 97-105.
20. **Zygner, W., Jaros, D., Wedrychowicz, H.,** Prevalence of Babesia canis, Borrelia afzelii and Anaplasma phagocytophilum infection in hard ticks removed from dogs in Warsaw (central Poland), Veterinary Parasitology, 2008, 153, 139-142.

OCCURRENCE AND MANIFESTATION OF STEREOTYPIC BEHAVIOUR IN CAPTIVE URSUS ARCTOS

LĂZĂRESCU C., CIOCONEA C., GAȘPAR C., GRIGOREANU A., ȚIBRU I.

Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645, Calea Aradului No. 119, Timisoara, Romania
E-mail: cristi_lazarescu@yahoo.com

Summary

The present study investigated the occurrence and manifestation of stereotypic behaviour in captive Ursus Arctos, the overall rate of displayed behaviour and the specific trigger that causes the manifestation. It is very well known the fact that an artificial environment can cause in different species abnormal behaviour during their captive life that can cause a decline in the welfare if the behaviour is not evaluated and corrected by specific measures. The results of this study showed that the occurrence of the abnormal behaviours is strongly linked to lack of environment enrichment and to an inadequate space allowance. The presence of stereotypic behaviour indicated poor welfare related to the inability to express normal behaviour.
Keywords: captive bears, ethogram, stereotypic behaviour.

Zoos offer unique research opportunities for many species of wild animals, some very difficult to access in natural conditions. In addition, the environment in captivity allows the control of variables (e.g. participation in breeding) that would be impossible to control in the wild. Also, zoos represent for students and young researchers true laboratories for the improvement of scientific techniques and for the preparation of scientific investigations in situ (10). The study of wildlife life in zoos is only possible on the basis of knowing how their lives unfold in freedom (2).

Zoos must contribute in particular to the protection of rare species, threatened with extinction, through the establishment of protected areas and other measures, including through assisted reproduction within the framework of programs of international interest (10).

In general, behaviour is the "first line of defense" of the animal in its response to changes in the environment. Careful observation of behaviour can provide us with an important package of information about requirements, preferences, inconveniences, and inner states, as a result of an interpretation that is firmly based on a knowledge of the behaviour al pattern of the species studied (9). The Zoo of Sibiu is a member of the Federation of Zoos and Aquariums in Romania (14), where the representatives of the zoos in the country meet periodically to establish collaboration relations (15). The Sibiu Zoo is located in the Natural Reserve "Dumbrava Sibiului Natural Park", being declared a protected area by Law No. 5 of March 6, 2000 (15).

According to the Constitution of the European Association of Zoos and Aquaria- EAZA, zoos are those permanent establishments open and managed to the public where wild animal species are kept in order to promote nature conservation in situ, by educating, informing and recreation of the public as well as facilitating research programs (7).

Ethology is a science that studies the biology of behaviour. Ethology emphasizes hereditary behaviour or instinct in its modern sense of a complex of motor reactions in a complex of motor reactions in a complex situation. The term ethology comes from the Greek language, being a word composed of: ethos (character) and logos (speech, science). Behaviour is the totality of observable activities in an animal (motors, attitudes, sound, chemical broadcasts, etc.), integrated and coordinated at the level of the organism, in response to sensory perceptions on the part of the environment, in order to survive and reproduce (5).

Materials and methods

In order to make the ethogram, the method of focal observation was used, in parallel with the audio-video recording. Two specimens of the Brown Bear species (Ursus arctos) located in the Sibiu Zoo, male (M1 and M2) were observed. The animals were subjected to observations between 7.40 and 20 o'clock, daily, for a period of 14 days.

To capture a wide range of behaviors, study periods ran over extended lengths of time at different times of the day. The total duration of the observation is 3094 minutes (51 hours and 34 minutes), the audio-video recording was made in parallel totaling 1768 minutes (29 hours and 28 minutes). The behaviour of the animals was noted, filmed, timed and framed in the appropriate behaviour al pattern. During the observation, the atmospheric conditions, details of the behaviour, the context in which they unfold and the possible unusual situations were noted.

At the end of the 14 days, the proportion of the main behaviours was calculated in relation to the total duration of the observation, and the time budgets for each copy were deducted, with the mention that on the 7th day of observation M1 was not subjected to observation after 8:28, being due to a treatment that required the use of a tranquilizing substance, after which he was allowed to rest in the shelter for the rest of the day.

Before the actual observations were gathered details on the history of the two bear specimens from the two veterinarians and the caregivers, this information played an important role in the process of interpreting the data. In order to draw up an etogram for the Brown Bear species, the behaviour was structured on three categories: solitary behaviour , behaviour related to food and social behaviour. Each category involves a series of behavioural patterns. For the etogram, they were used

as materials, notebook, pen, audio-video camera, tripod, mobile phone and stopwatch.

Behaviour, like any characteristic characteristic of a living system, has a hereditary determinant and another acquired, but the degree to which the various qualities, which define a behaviour al act, are inherited or learned, presents a register of variation with extremely wide limits (3).

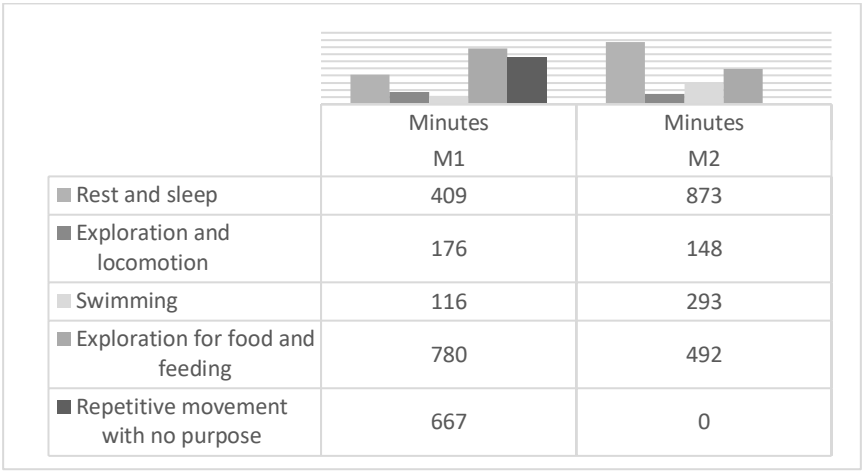
Results and discussions

The context in which the conflicts appear is given by the presence of bears in a small space. The completion of this aggressive behaviour was almost every time followed by the withdrawal of one of the bears. The exception being the confictul generated by the fact that the two specimens were kept together in a small space, longer than usual (over 4 hours), which led to a more aggressive behaviour , resulting in physical contact. Careful observation of behaviour can provide us with an important package of information about requirements, preferences, inconveniences, and inner states, as a result of an interpretation that is firmly based on a knowledge of the behaviour al pattern of the species studied (9).

Regarding the behaviour related to food, given that the food is administered daily, at 8 o'clock, following the observations made, it resulted that depending on the way in which the animals receive the food, this infuriates other behaviours (13). If the placement of food is made on a larger area, the animals require more time to find it, which leads to a more pronounced expression of the exploratory behaviour. Observations also show that the M1 requires a longer duration to feed, and the expression over a longer period of time of the exploratory behaviour in the sense of searching for food, leads to the elimination of stereotypical behaviour. The mental state depends on the existence and the categories of stimuli in the external and internal environment, which can be perceived differently, depending on the experience of each animal (4).

With reference to the manifestation of bathing behaviour (aquatic locomotion) (12), it resulted from the observations that the duration of the expression of this behaviour is almost double in the M2 copy compared to the M1 specimen. Also, the duration of bathing periods is on average 10 minutes, with a frequency of three, four times a day. In bathing behaviour, the expression of other behaviours such as: body care behaviour, play behaviour, as well as feeding behaviour (8), the animals carrying fruits and vegetables to the pool, was also observed.

Following the observations made and the calculation of the time budgets, it can be inferred that the two bear specimens express a different behaviour, in the context in which the two bears are brothers, and have lived together in the same living environment for 20 years (Fig. 1).



	Minutes M1	Minutes M2
Rest and sleep	409	873
Exploration and locomotion	176	148
Swimming	116	293
Exploration for food and feeding	780	492
Repetitive movement with no purpose	667	0

Fig. 1. Behaviours expressed in minutes of both individuals

It can also be argued that M2 prefers to rest in a proportion of 28% compared to M1 which allocates to the behaviour of yesteryear and sleep a percentage of only 14%. Observations show that M1 is much more active than M2, and devotes much more time to food-related behaviour.

During the observation period, M1 frequently manifested repetitive locomotion movements, without a certain completion, over a distance of 3-4 meters, along the fence, a stereotypic behaviour common in bears in captivity (11), which occurs as a result of the animal's impossibility to explore a larger territory. The stereotypical behaviour represented a high percentage of 22% of the total duration of the observation, being observed over a number of 12 days.

Following the observations made, the playful behaviour (1) is expressed in the two bears most often during the nesting spent in the two pools arranged within the pen.

With reference to the agonistic behaviour (13), the observations showed that a strict hierarchy (6) between the two bears is not established, in the sense that there is no dominant specimen and a dominated one, the conflicts being generated in certain specific situations, by both specimens. At the beginning of the observation period it was M1 who initiated the attack and M2 withdrew, and in the second part of the ethological study, it was M2 who attacked more often and M1 withdrew first. This behaviour was observed within 7 days of the 14 days of the study.

Conclusions

As a result of the observations carried out, it resulted that the two bears, although they were kept for five months in inappropriate conditions, after being reinstated in the new habitat, they did not change their initial major behaviour al patterns, showing a normal behaviour. Agonistic behaviour occurred frequently at times when the two specimens were kept in a confined space. Following the observation and calculation of the time budgets, the two copies have different behaviour al patterns.

References

1. **Clapham, M., Kitchin, J.,** Social play in wild brown bears of varying age-sex class, Acta Ethologica, 2016, 19, 3, 181-188.
2. **Cociu, M.,** Viaţa în zoo. Introducere în biologia captivităţii, Ed. Ştiinţifică şi Enciclopedică, Bucureşti, 1980.
3. **Coman, I., Podoleanu, L., Gaşpar, C.,** Etologie şi etopatologie, Ed. Tehnopress, Iaşi, 2002.
4. **Decun, M.,** Bioetica, bunăstare şi protecţia animalelor, Ed. Mirton, Timişoara, 2013.
5. **Decun, M.,** Etologia, bunăstarea şi protecţia animalelor, Ed. Mirton, Timişoara, 2004.
6. **Erina, K., Adamič, M.,** Fifty Years of Brown Bear Population Expansion: Effects of Sex-Biased Dispersal on Rate of Expansion and Population Structure, Journal of Mammalogy, 2008, 89, 6, 1491-1501.
7. **European Associaion of Zoos and Aquaria (EAZA)** standards for the accomodation and care of animals in zoos, 18/06/94.
8. **Grandia, P.A., Jiska, J.vD., Paul, K.,** Stimulating Natural Behaviour in Captive Bears, Ursus, 2001, 12, 199-202.
9. **Joy, M.,** Why It Is Important to Understand Animal Behaviour, ILAR Journal, 1998, 39, 1, 20-26.
10. **Rusu, A.S.,** Principii moderne de amenajare a grădinilor zoologice, Ed. Presa Universitară Clujeană, Cluj-Napoca, 2008.
11. **Sahlén, V., Ordiz, A., Swenson, J.E., Støen, O.G.,** Behavioural Differences between Single Scandinavian Brown Bears (Ursus arctos) and Females with Dependent Young When Experimentally Approached by Humans, PLoS One, 2015, 10, 4, e0121576.
12. **Soriano, A.I., Vinyoles, D., Maté, C.,** Abnormal Behaviours in Two Captive Brown Bear (Ursus arctos Linnaeus, 1758) Females: Individual Differences and Seasonal Variations, Der Zoologische Garten, 2017, 86, 1-6, 88-101.

13. **Zarzo-Arias, A., Delgado, M. del M., Ordiz, A., García Díaz, J., Cañedo, D., González, M.A., Penteriani, V.,** Brown bear behaviour in human-modified landscapes: The case of the endangered Cantabrian population, NW Spain, Global Ecology and Conservation, 2018, 16, e00499.
14. ***http://federatiazoo.ro/cine-suntem
15. ***https://ro.wikipedia.org/wiki/Rezerva%C8%9Bia_natural%C4%83_%22Parcul_Natural_ Dumbrava_Sibiului%22

A RETROSPECTIVE STUDY OF THE CONGENITAL MALFORMATIONS IN DOGS AND CATS (2019-2021)

MARC S., SPĂTARU I.I., MIRCU C., TORDA I., OTAVĂ G.

Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645, Calea Aradului No. 119, Timisoara, Romania
E-mail: simona.marc@usab-tm.ro

Summary

Congenital malformations are considered structural and functional defects of different regions of the body that occur during embryogenesis and are seen immediately after parturition. The causes can be genetic, environmental or a combination of both. Most of these malformations have a genetic predisposition due to genetic mutations that are transmitted between generations. The mutant gene can accumulate in the population through various ways such as: the existence of carriers, selection for a specific character that can be related to deleterious genes, genetic drift or even no selection against abnormal phenotypes. The present study aims to centralize the incidence of congenital malformations in dogs and cats encountered at the Small Animal Reproduction Clinic, FMV Timisoara, between 2019-2021. The results obtained after the examination of 294 animals were the identification of 29 cases (9.86%) with congenital malformations, of which 23 cases (7.82%) in dogs and 6 cases in cats (2.04%). It was observed that in purebred animals the frequency of birth defects was higher, so 86.96% of affected dogs and 66.67% of affected cats were purebred. Among the clinically congenital malformations identified we mention: hydrocephalus, lip cleft, palate cleft, cheilognatopalatoschisis, heterochromia, polydontia, syndactyly, polymastia or albinism. Most malformations are congenital, but there are also cases in which the defects are visible after a few months postpartum or even years later. Regardless of whether it is pure bred or mixed breed, it is important to have a correct diagnosis through genetic tests or pedigree, when available, to identify the carriers, to improve the veterinary care and finally to make a genetic advice to the breeders in order to increase the health of the animals of the future generations.
Keywords: congenital malformations, gene, carrier, purebred, mixed breed.

According to the World Small Animal Veterinary Association, there are approximately 900 hereditary diseases and genetic predispositions in dogs and 200 in cats (25). Many of the genetic diseases are known to have a heritable basis and have started to play an increasingly important role in veterinary medicine.

Knowledge of the genetic epidemiology of diseases inherited from carnivores is very important for veterinary care, but also for breed clubs or dogs and cats organizations with the aim of establishing guidelines for good animal breeding practices. This knowledge is important for both purebreds and mixed breeds, as their percentage in the dog population is increased (5). The estimated percentage of

mixed dog breeds in the United States is 53% according to the American Veterinary Medical Association, in Germany and the United Kingdom about 31-33% and in Australia about 50% (19).

An extensive study from 2018 done one 96.673 dogs that were genotyped (83.220 mixed breed and 13.453 purebred dogs) found that mixed breeds were 1.6 times more susceptible than purebreds to be a carriers for at least one of the 9 recessive disease variants studied (30.3% vs. 18.4% of dogs, respectively). When the number of homozygous recessive diseases was taken into account, purebred dogs were 2.7 times more likely than mixed breed dogs to be genetically affected for at least one of the common recessive disorders (5).

Genetic diseases that comply with Mendelian lows of heredity (Mendelian disorders) are important in the dog population, because the frequency of carriers is quite high, about 10-30% (18).

The variety of dog breeds is about 500 (12) and 40-71 cat breeds (1), a variety that has been achieved over the years through the directed selection for certain morphological, but also behavioral traits. In cats, the selection of phenotypes is related to the coat or to the morphology of the body, traits that were pleased to breeders (1).

The selection of characters of genetic origin and the reduction in the number of ascendants have led to an increase in homozygous population, which often leads to a loss in biological fitness and an increase in the probability of the existence of descendants affected by deleterious or recessive alleles for disease-causing (2, 8). The specifications of pedigree / purebred dogs are defined by the organizations that register and credit these animals, for example Kennel Club from the UK, Kennel Club from America, the Federation Cynologique Internationale or others. Dogs can only be registered if both parents are registered. This reproductive isolation given by the "breed barrier" rule can lead to a reduction in genetic diversity within the breed and possibly a promotion of breed specific genetic diseases (12). A study of genetic diversity among 13 purebred dog populations in the UK found a high degree of homozygosity compared to crossbred animals. The Boxer and West Highland white terriers had the lowest heterozygosity. The Rottweiler and Golden Retriever breeds had the highest level of inbreeding (12).

Genetic diversity of a population can be measured by genetic heterozygosity tests based on single nucleotide polymorphisms (SNP) genotyping. These SNPs can estimate the genetic relationship between individuals. Heterozygosity ensures an increase in the adaptability of animals to new environmental conditions. These genome-wide SNP testing tests are more accurate than pedigree or short-tandem repeat molecular markers. Hererosity tests and DNA panel screening for known or other inherited genetic diseases that have not been related to the respective breed offer the possibility of developing a correct reproduction strategy by excluding unknown variants of the disease that could spread unintentionally in the population (8). In cats, for example, 63K Feline SNP array dataset from 26 cat breeds and with the help of a genetic differentiation-based method (di), potential candidate genes for

several phenotypes were identified (DLX6, DLX5, DLX2 gene – for brachycephaly; MCRIP2 and PBX1 gene for curled ears of American Curl or ADGRD1 gene for body-form of Siamese and Oriental cat (1).

The present study aims to quantify the congenital malformations found in dogs and cats according to breed, cases encountered over three years, in the Small Animal Reproduction Clinic of the Faculty of Veterinary Medicine from Timisoara.

Materials and methods

The study was carried out on 164 dogs and 130 cats that were presented to the Small Animal Reproduction Clinic from Faculty of Veterinary Medicine, Timisoara, between 2019-2021, for various pathologies of the reproductive system or for C-section. Congenital abnormalities were identified during the clinical examination of newborns obtained by C-section or of adult animals. The animals examined were purebred and mixed breed.

Results and discussions

The clinically congenital malformations identified in dogs and cats were: hydrocephalus, cleft lip, cleft palate, cleft lip and jaw, heterochromia, polydontia, syndactyly, polymastia, albinism, polydactyly, dewclaw (hind limb specific preaxial polydactyly), primary glaucoma, congenital cataracta, congenital phimosis and epitheliogenesa imperfecta (Fig. 1 and Fig. 2).

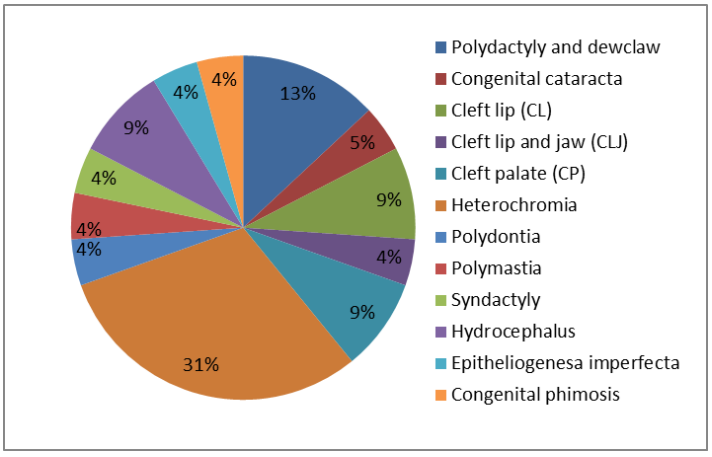


Fig. 1. The congenital malformations identified in dogs (%)

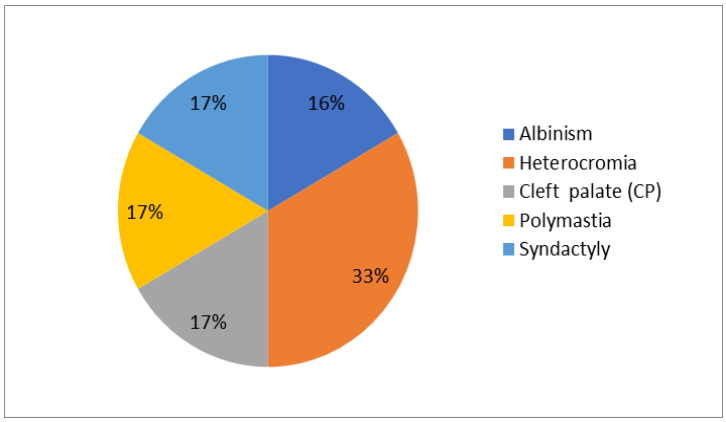


Fig. 2. The congenital malformations identified in cats (%)

The results obtained after the examination of 294 animals were the identification of 29 cases (9.86%) with congenital malformations, of which 23 cases (7.82%) in dogs and 6 cases in cats (2.04%) (Fig. 3). It was observed that in purebred animals the frequency of birth defects was higher, so 86.96% of the affected dogs and 66.67% of the affected cats were purebred (Fig. 4).

Most of these malformations in dogs were with non-syndromic evolution (91.3%), but there were also situations with a syndromic evolution (8.7%). In cats, all 6 cases of congenital malformations were with nonsyndromic evolution. The dog breeds with congenital malformations were Beagle, Chihuahua, Golden Retriver, African Mastiff, French Bulldog, Small Schnautzer, German Shorthair Pointer and in cats: Sfinx, Scotish fold and Persian breed (Fig. 5).

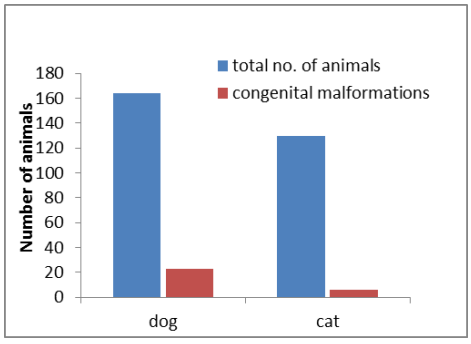


Fig. 3. Number of animals with congenital malformation

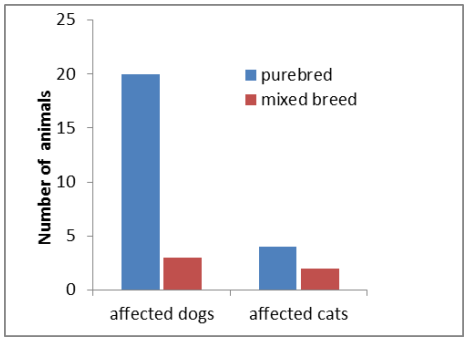


Fig. 4. Number of purebred and mixed breeds with congenital malformation

Heterochromia was the defect most frequently seen (31%) in dogs and cats (33%) from our study. This pathology is defined by the existence of eyes with different colors, an aspect that is often due to genetic mutations that reduced melanin synthesis or affect melanocyte and melanosome function in the epithelium of the retinal pigment. Blue eyes are representative of Siberian Husky and factors that produce blue eyes are associated with Merle and Piebald dogs, where Premelanosome Protein (PMEL17) gene and Melanogenesis Associated Transcription Factor gene (MITF), the master regulator of melanocyte development, lead to complete or sectorial heterochromia associated with depigmented regions across the face (4). Also simple repeat polymorphism in MITF gene affects promoter activity thus being a key regulator of white spotting in dogs, because the white spotting locus in dogs is colocalized with the MITF gene (10). A large study carried out on more than 3000 dogs showed that a region of canine chromosome 18 carrying a tandem duplication near the ALX4 gene is strongly associated with the variation in blue eye color (4).

Another congenital malformation seen in 9% of the 23 dogs studied was hydrocephalus, a neurologic disorder characterized by an excessive accumulation of cerebrospinal fluid (CSF) in the brain ventricles and arachnoid cavity caused by excessive production or inadequate re-absorption into the bloodstream by blockage in the ventricular system. The accumulation of CSF enlarges the brain leading to increased pressure in the skull, followed by thinning of the cranial bones and compression of the brain including brain atrophy and a variety of symptoms, some of which may be life threatening (17). In puppies, this is generally a genetic problem related to the breed. The breeds with a high prevalence in hydrocephalus are those with a anatomically tendency to a domed-head like Chihuahua, Pomeranian, English Bulldog, Shih-Tzu, King Charles Spaniel, Boston Terrier and in cats some evidence had been found in Siamese, Persians and Manx cats (6).

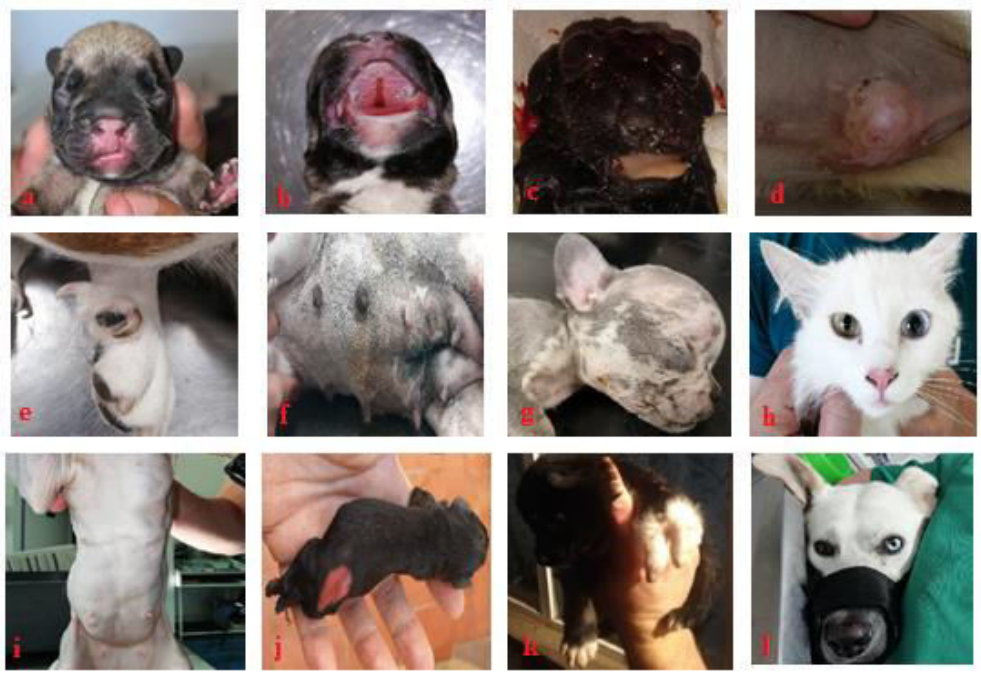


Fig. 5. Clinical appearance of some congenital malformations seen in dogs and cats (a. Lip cleft, b. Palate cleft, c. Primary glaucoma, d. Congenital phimosis, e. Hind limb specific preaxial polydactyly, f.&i. Polymastia, g. Hydrocephalus, h.&l. Heterochromia, j. Epitheliogenesa imperfecta, k. Syndactyly

Congenital defects in the oral cavity, such as cleft lip (CL), cleft lip and jaw (CLJ), or cleft palate (CP) were observed in 21.74% from dogs with abnormalities and 16.70% of cats with abnormalities. The CL/CLJ/CP are developmental disorders due to failure of the frontonasal, medial nasal processes, lateral nasal processes, maxillary or mandibular processes to close (3, 14, 15, 20). In dogs, the cleft lip and palate are the most common birth defects, due to breeding practices. CL or CP can be caused by maternal exposure to environmental factors such as excessive doses of vitamin A (125 000UI/kg BW at 17-22 days of gestation), aspirin (400 mg/kg/day at 23-30 days of gestation), various substances such as griseofulvin, anabasine, metronidazole, primidone, suplhanamides, 6-diazo-5-oxo-L-norleucine (DON), viral infections, cytostatic drugs, stress or hormonal factors (11, 15). The teratogenic effects of some substances depend on the species, the dose, duration of action and the stage of development of the embryo (15). Because certain breeds of dogs are more likely to have CL-CP, especially brachycephalic dogs, indicates a strong contribution of genetic factors. For example in Boxers the frequency of CP is 0.6%, in Beagle 0.11%, in Pyrenees Shepherd dogs 2.2% (9, 11). Some candidate genes

associated with CP inherited with an autosomal recessive pattern in dogs are TGFb3, MSX1, FST, DLX5 or DLX6 (11).

Congenital phimosis found in a French Bulldog is considered a developmental anomaly of the penis and prepuce, clinically seen as an small orifice and entrapment of the penis inside the prepuce. It has been identified in some dog breeds, such as German shepherds, Golden retrievers, Bouvier des Flandres or Labrador retrievers, but also in cats and stallions (26). The cause in congenital phimosis is not known, but for acquired form the most common causes are neoplasia, edema, trauma, inflammation, sucking by littermates or even licking by the dam (24). There are various surgical techniques to correct this defect, but if it is a congenital form, castration should be considered (21, 24).

Polydactyly is another embryonic developmental disorder found in a dog, characterized by abnormal number of digits. One type of polydactyly in dogs is dewclaw or hind limb specific preaxial polydactyly (PPD), a dominant genetic mutation (16). Molecular analyzes identified the cause of polydactyly as point mutations in the ZPA regulatory sequence (ZRS) found in intron 5 of the LMBR1 gene. The LMBR1 gene (limb development membrane protein 1) is located approximately 1 Mb away from the Shh gene (required for proper anterior/posterior pattering) which is expressed in the mesenchymal tissue on the posterior edge of the limb, in the region called ZPA (the zone of polarizing activity). The regulation of Shh gene expression is performed by ZRS. The linkage between the Shh gene and ZRS is well preserved in mammals, and mutations in one of the two genetic regions affect the function of the other (16). The Lundehund dogs, characterized by unique joint flexibility and polydactyly on all limbs, have the mutant genotype for LMBR1 gene, an example that mutations in LMBR1 gene can cause polydactyly. Also due to the low genetic variability of this breed, they can develop the Lundehund syndrome, a severe gastro-enteropahtic disease caused by a missense mutation in the LEPRE1 gene: g.139212C> G (13).

Albinism was seen in a cat from our retrospective study. This congenital disorder is characterized by lack of pigment in hair, skin and eyes. In cats the mutations in the TYR gene (tyrosinase) are responsible for the disease, similar in mice, humans, rabbits or cattle. In cats, the TYR gene corresponds to the colour locus, its alleles, from dominant to recessive are: C (full colour) > c^b (burmese) > C^s (siamese) > c (albino) (7). In dogs, different mutations in SLC45A2 (solute carrier family 45, member 2) gene are implicated in albinism, in an albino Lhasa Apso a deleterious substitution, p.G493D, was confirmed (22).

Polymastia observed in one bitch and one cat is a minor hereditary disease whose manifestation consists in the existence of supernumerary teats compared to the one characteristic of the breed and the species. The mutant gene is responsible for the growth of supernumerary mammary buds along the milk line during embryonic development. If the process is not disturbed the phenotypic expression will be of the complete mammary gland (parenchyma and teats), but sometimes the process is

disrupted and then the expression will be incomplete, only the teats will be supernumerary, without functional mamary parenchyma (polythelia) (3).

In conclusion, modern molecular investigations provide “big data” to guide accurate diagnostic tests in hereditary diseases to identify sick animals, carrier ones, in order to be able to do genetic counseling of the animal breeder or to do breed selection. Veterinary education and breed health research are important for improving the genetic health of future generations (5, 23, 25).

Conclusions

The frequency of congenital defects in purebred animals was higher, so 86.96% of the affected dogs and 66.67% of the affected cats were purebred. Heterochromia was the defect most frequently seen (31%) in dogs and cats (33%) from our study.

Most congenital defects are clinically identified, but in order to know the cause of these changes during embryonic development and to exclude external factors, it is important to perform genetic tests, when available, genetic tests that we intend to use in the future.

Regardless of whether it is pure bred or mixed breed, through genetic tests or pedigree, when available, carriers of undesirable genes can be identified and in this way it can be improved the veterinary care and finally to make a genetic advice to the breeders in order to increase the health of the animals of the future generations.

References

1. **Alhaddad, H., Abdi, M., Lyons, L.A.,** Patterns of allele frequency differences among domestic cat breeds assessed by a 63K SNP array, PLoS One, 2021, 16, 2, e0247092.
2. **Boccardo, A., Marelli, S.P., Pravettoni, D., Bagnato, A., Busca, G.A., Strillacci, M.G.,** The German Shorthair Pointer Dog Breed (Canis lupus familiaris): Genomic Inbreeding and Variability, Animals, 2020, 10, 498.
3. **Bonca, G, Marc, S.,** Boli genetice la animale si pasari, Ed. Mirton, Timisoara, 2020.
4. **Dean-Coe, P.E., Chu, E.T., Slavney, A., Boyko, A.R., Sams, A.J.,** Direct to consumer DNA testing of 6000 dogs reveals 98.6-kb duplication associated with blue eyes and heterochromia in Siberian Huskies, PloS Genetics, 2018, 14, 10, e1007648.
5. **Donner, J., Anderson, H., Davison, S., Hughes, A.M., Bouirmane, J., Lindqvist, J., Lystle, K.M., Ganesan B., Ottka, C., Ruotanen, P., Kaukonen, M., Forman, O.P., Fretwell, N., Cole, C.A., Lohi, H.,** Frequency and

distribution of 152 genetic disease variants in over 100,000 mixed breed and purebred dogs, PLoS Genetics, 2018, 14, 4, e1007361.

6. **Estey, C.M.,** Congenital Hydrocephalus, Veterinary Clinics of North America Small Animal Practice, 2016, 46, 2, 217-229.
7. **Imes, D.L., Geary, L.A., Lyons, L.A.,** Albinism in the domestic cat (*Felis catus*) is associated with a tyrosinase (TYR) mutatian, Animal Genetics, 2006, 37, 2, 175-178.
8. **Keijser, S.F.A., Fieten, H., Vos-Loohuis, M., Piek, C.J., Anderson, H., Donner, J., Scholten, I., Nielen, M., Hesselink, J.W., van Steenbeek, F.G.,** Heterozygosity testing and multiplex DNA panel screening as a potential tool to monitor health and inbreeding in a small, closed dog population, Canine Genetic Epidemiology, 2018, 5, 12.
9. **Kemp, C., Thiele, H., Dankof, A., Schmidt, G., Lauster, C., Fernahl, G., Lauster, R.,** Cleft Lip and/or Palate with Monogenic Autosomal Recessive Transmission in Pyrenees Shepherd Dogs, The Cleft Palate-Craniofacial Journal, 2009, 46, 1, 81-88.
10. **Korberg, I.B., Sundstrom, E., Meadows, J.R.S., Pielberg, G.R., Gustafson, U., Hedhammar, A., Karlsson, E.K., Seddon, J., Soderberg, A., Vila, C., Zhang, X., Akesson, M., Lindblad-Toh, K., Andersson, G., Andersson, L.,** A simple repeat polymorphism in the MITF-M promoter is a key regulator of white spotting in dogs, PLoS One, 2014, 9, 8, e104363.
11. **Lobodzinska, A., Gruszczynska, J., Max, A., Jan Bartyzel, B., Mikula, M., Mikula Jr., I., Grzegrzolka, B.,** Cleft palate in the domestic dog *Canis Lupus* Familiaris- etiology, pathophtsiology, diagnosis, prevention and treatment, Acta Scientiarum Polonorum Zootechnica, 2014, 13, 3, 5-28.
12. **Mellanby, R.J., Ogden, R., Clements, D.N., French, A.T., Gow, A.G., Powell, R., Corcoran, B., Schoeman, J.P., Summers, K.M.,** Population structure and genetic heterogeneity in popular dog breeds in the UK, Veterinary Journal, 2013, 196, 1, 92-97.
13. **Metzger, J., Pfahler, S., Distl, O.,** Variant detection and runs of homozygosity in next generation sequencing data elucidate the genetic background of Lundehund syndrome, BMC Genomics, 2016, 17, 535.
14. **Moura, E., Cirio, S.M., Pimpão, C.T.,** Nonsyndromic Cleft Lip and Palate in Boxer Dogs: Evidence of Monogenic Autosomal Recessive Inheritance, Cleft Palate-Craniofacial Journal, 2012, 49, 6, 759-760.
15. **Moura, E., Turra Pimpao, C.,** Cleft lip and palate in the dog: medical and genetic aspects, Intechopen, 2017.
16. **Park, K., Kang, J., Subedi, K.Pd., Ha, J.H., Park, C.,** Canine polydactyl mutations with heterogeneous origin in the conserved intronic sequence of LMBR1, Genetics, 2008, 179, 2163-2172.

17. **Schmidt, M., Ondreka, N.,** Hydrocephalus in Animals, Pediatric Hydrocephalus, 2019, 53-95.
18. **Seppälä, E.H., Jokinen, T.S., Fukata, M., Fukata, Y., Webster, M.T., Karlsson, E.K., Kilpinen, S.K., Steffen, F., Dietschi, E., Leeb, T., Eklund, R., Zhao, X., Rilstone, J.J., Lindblad-Toh, K., Minassian, B.A., Lohi, H.,** LGI2 Truncation Causes a Remitting Focal Epilepsy in Dogs, PLoS Genetics, 2011, 7, 7, e1002194.
19. **Turcsan, B., Miklosi, A., Kubinyi, E.,** Owner perceived differences between mixed-breed and purebred dogs, PLoS One, 2017, 12, 2, e0172720.
20. **Usui, K., Tokita, M.,** Creating diversity in mammalian facial morphology: a review of potential developmental mechanisms, EvoDevo, 2018, 9, 15.
21. **Vadalia, J.V., Patel, A.M., Kumar, V.,** Surgical management of congenital phimosis in pup, Indian Journals - Intas Polivet, 2014, 15, 1, 160-162.
22. **Wijesena, H.R., Schmutz, S.M.,** A Missense Mutation in SLC45A2 Is Associated with Albinism in Several Small Long Haired Dog Breeds, Journal of Heredity, 2015, 106, 3, 285-288.
23. **Wolf, Z.T., Brand, H.A., Shaffer, J.R., Leslie, E.J., Arzi, B., Willet, C.E., Cox, T,C., McHenry, T., Narayan, N., Feingold, E., Wang, X., Sliskovic, S., Karmi, N., Safra, N., Sanchez, C., Deleyiannis, F.W.B., Murray, J.C., Wade, C.M., Marazita, M.L., Bannasch, D.,** Genome-Wide Association Studies in Dogs and Humans Identify *ADAMTS20* as a Risk Variant for Cleft Lip and Palate, PLoS Genetics, 2015, 11, 3, e1005059.
24. **Yoon, H.Y., Jeong, S.W.,** Surgical correction of a congenital or acquired phimosis in two cats, Journal of Veterinary Clinics, 2013, 30, 2, 123-126.
25. ***https://wsava.org/committees/hereditary-disease-committee, World Small Animal Veterinary Association, Hereditary Disease Commitee.
26. ***https://www.dvm360.com/view/challenging-case-phimosis-young-adult-dog, Saporitio, K., A challenging case: phimosis in an young adult dog.

MICROBIOLOGICAL AND SENSORY INDICES OF DOMESTIC RABBIT MEAT

MARDARI T., GOLBAN R.

The State Agrarian University of Moldova,
42 Mircești Street, Chișinău, Republic of Moldova
E-mail: golbanrita@gmail.com

Summary

The paper presents the results obtained from the assessment of sensory and microbiological indices of rabbit carcasses of different origins. The studied carcasses were purchased from a specialized store, from the market and from a farm. The obtained data indicate an increased bacterial load in the carcass from the market both on the surface and in depth, and the carcass from the specialized store fell into the category of fresh meat according to sensory and micro biological indices.

Keywords: rabbit meat, microbiological indices, microflora.

Rabbit meat is very good for the human diet, due to the fact that its fat is not deposited on human muscle fibers; it contains such minerals as zinc, sodium, potassium, calcium, magnesium and iron (2, 6).

Rabbit meat has the fewest calories, for example 450 g of homemade rabbit meat has 795 calories, and the same amount of chicken contains 810 calories (3, 4).

The consumption of rabbit meat varies a lot from one country to another, from one region to another, it is quite appreciated in France, Holland, England, Austria, Italy, but if compared to the Republic of Moldova the consumption of rabbit meat is quite low here (1, 7).

The human body assimilates protein from rabbit meat in a proportion of approx. 90%, at the same time it assimilates beef protein only up to 62% (5, 8).

Due to its complex composition, rabbit meat is also a favorable environment for the intense development of various microorganisms harmful to consumer's health (10, 14).

The following groups of dangerous pathogens can be found in rabbit meat: viral, bacterial, parasitic (9, 11).

At a correct cooling and maturation regime, a dry film is formed on the meat surface that prevents the microorganisms from penetrating the meat (13, 15).

The maximum term for keeping the carcass / sliced meat, refrigerated and stored at t0 = + 2 + 4 0C is for rabbit meat - 5 days, according to GD no. 696 of 04.08.2010 (17, 19).

The evaluation of rabbit carcasses quality is done subjectively and objectively.

Subjective evaluation is based on the appearance, the colour of the meat in the carcass, the state of fattening. On the outside, the carcass must not have any ruptured tissues, any hairs, traces of blood or intestinal contents. The colour of the

meat must be pale pink, and the state of fattening must be followed by the uniformity of the fat layer on the surface of the carcass.

The objective evaluation is made according to the meat weight and quality. The quality of the meat is assessed through chemical and bacteriological examination. Chemical control should not exceed 32 mg per 100 g meat of nitrogen in hydrolysis. Bacteriological examination means taking smears (maximum 3-4 cocci are allowed per field). Meat containing pathogenic or facultative pathogenic bacteria is not produced and marketed (20).

The criteria for the sensory evaluation in establishing meat freshness are: the external appearance, colour, consistency, odour and appearance of the bone marrow. According to the evaluation, meat can be: fresh, relatively fresh and spoiled (16, 18).

The bacterial load on the meat surface increases progressively, depending on the temperature, the duration and the storage conditions of the meat (12, 14).

By following the hygiene steps when slaughtering animals, the risk of meat contamination is reduced by over 90%, these steps are: strictly healthy animals, observance of hygienic norms, equipment cleaning, water quality, etc.

Thus, the purpose of the investigations is to study sensory and microbiological indices of commercial rabbit meat and carcasses and to assess compliance with the rules in force.

Materials and methods

The biological material used was rabbit carcasses from different sources:

- the rabbit carcass purchased from a specialized shop;
- the carcass purchased from the central market in a simple hall arranged for the sale of the meat of different species of animals;
- the carcass procured from a farm which raises rabbits for family purposes.

In order to obtain a conclusive result, the work instructions according to GOST 26668-85 regarding the collection of meat samples were observed, as the conclusion of the laboratory examination depends on this operation.

The research was carried out in the microbiology and immunology laboratory of the Faculty of Veterinary Medicine.

In laboratory conditions, some sensory and microbiological indices of rabbit carcasses and meat were evaluated.

Results and discussions

The sensory indices taken into account when evaluating meat are perceived with the help of the sense organs: colour, smell, consistency and taste. The sensory properties of meat depend on its structure and composition, on the mode and physiological state of the animal at the time of slaughter, the pathological condition and the state of freshness.

The colour of the meat is influenced by the colour of the muscle fibers varying from one colour to another. The colours can be from white to grey, the coloration in red with different shades depends on the content of myoglobin and haemoglobin, to which is added the colour of the connective and adipose tissue. Meat colour can change when contacting with air, or by the way it was obtained.

Normal meat has an elastic, fine and juicy consistency.

The odour of the meat differs from one species to another; odour evaluation is made on fresh meat. The odour depends on the diet, the age of the animal, the physiological condition, etc.

The taste of the meat is influenced by the tenderness, fineness, juiciness of the muscle fibers, the distribution of fat and the quality of the feed.

The studied rabbit carcasses were assessed for the determination of freshness, and the results are presented in the table (Table 1).

Table 1

The results of the sensory evaluation of rabbit carcasses

Studied indices	**Origin of the studied carcasses**		
	from a specialized meat store	**from the Central Market**	**from the farm**
Exterior appearance	The surface of the meat has a dry film, normal consistency, characteristic of the rabbit species	Dry surface, unpleasant appearance of old meat, matte meat, rancid odour	Wet surface, with partially sticky mucus, fat with a matte appearance
Colour	Pink-red specific to the species, the section is glossy and slightly moist	The surface of the carcasse is grey	Light pink, moist
Consistency	It is firm and elastic, no hardening is formed when pressed with the finger.	When pressed with the finger shallows appear on the surface which doesn't return to its original state	Soft to the touch, when pressed with the finger, the meat returns to its original state
Odour	Pleasant and characteristic of the rabbit species	Unpleasant, stale	Slightly acidic, with a strong odour of unventilated meat
Freshness degree	Fresh	Altered	Relatively fresh

Having carried out the sensory evaluation of the rabbit carcasses from various places, we have noticed that the carcass purchased from the specialized store according to the degree of freshness can be included in the fresh category, the carcass from the farm- relatively fresh, and the one from the market was altered.

The quality of any meat is influenced by the number of microorganisms and their type. This is due to non-compliance with hygiene requirements during slaughter, processing or handling of carcasses. The presence of bacteria in meat limits its shelf life, and if there are also pathogenic germs then they endanger the consumer's health.

In conditions of temperature and high humidity above the permissible limits, the meat is spoiled. Depending on the type and mode of meat alteration, aerobic and anaerobic bacteria are distinguished.

The longer it takes for the animal to be slaughtered, the greater is the chance of microbes multiplying and penetrating.

Aerobic bacteria grow on the meat surface, they multiply in the connective tissue, and then they attack the muscle fibre. As degradation progresses, anaerobic bacteria appear.

Following the microbiological study, the sold meat is divided into 3 categories:

- fresh meat, where the microflora constitutes up to 10 cocci,
- meat of dubious freshness, where the bacterial microflora constitutes more than 30 cocci under microscope
- altered meat, where the bacterial microflora constitutes more than 30 cocci, and some rod shaped bacteria are also present.

The results of the microbiological study of the studied rabbit carcasses are reflected in the following tables.

The data from the table indicate some quantitative aspects of the bacterial microflora of the number of colonies on agar plates, thus we can observe that there were detected in the superficial layer of the carcass approx. 80 bacterial colonies of microflora for the rabbit carcass purchased from the farm, 75 colonies for the rabbit carcass from the market and 7 colonies for the carcass from the store, respectively. In depth, 35 colonies were found in the carcass from the farm, 31 in the carcass from the market and one colony in the carcass from the store (Table 2).

In the agar test tubes, the number of observed colonies was higher in the carcass from the farm - 30, in the carcass from the store - 10, and in the carcass from the market - 3 bacterial colonies.

Following the examination of microbial smears, the number of microorganisms on microscopic field was determined in (Table 3).

Thus we can notice that: in the carcass from the market, the superficial microflora constituted 70 bacteria (cocci, gram positive) on the microscopic field and in the other carcasses there were 30 colonies in each. The microflora in the depth also denotes a larger amount of bacteria in the market carcass - 50 colonies on the microscopic field.

Table 2

Comparison of the amount of bacterial microflora by culture media of rabbit meat according to origin, colonies

Culture media		Carcass from the store		Carcass from the market		Carcass from the farm	
		Microflora		Microflora		Microflora	
		superficial	depth	superficial	depth	superficial	depth
Plates	agar	7	1	75	31	80	35
	endo	0	0	0	0	0	0
Tubes	agar	10	1	3	1	30	12
	broth cultural aspects	turbidity, sediment	film, clear ring	turbidity, sediment	film, clear ring	turbidity, sediment	film, clear ring

Table 3

Comparison of the amount of bacterial microflora on smears of rabbit meat by origin, colonies

On the microscopic field	Carcass from the store		Carcass from the market		Carcass from the farm	
	Microflora		Microflora		Microflora	
	superficial	depth	superficial	depth	superficial	depth
Number of microorganisms	30	10	70	50	30	23
Bacterial morphological aspects	Cocci, gram positive	Cocci, gram positive	Cocci, gram positive	Cocci, gram positive	Cocci, gram positive	Cocci, gram positive

These aspects indicate that the rabbit carcass purchased from the specialized store is fresh compared to the other studied carcasses, which indicated a dubious or obsolete freshness.

Conclusions

According to the carried out investigations, we can draw the following conclusions:

The studied rabbit carcasses showed a varied number of coccyx microorganisms both in the surface layers and in the depth of the meat depending on the origin.

The rabbit carcass purchased from the specialized store falls into category I by freshness; the other carcasses have a dubious or obsolete freshness.

The number of microbial colonies detected in the studied carcasses varied, the carcass from the store contained 7 colonies, the one from the market - 75 colonies and the carcass from the farm - 80 colonies on the superficial surface of the carcass.

The microbial colonies detected in the depth of the meat of the studied carcasses are in smaller quantity, namely, in the carcass from the store -1 colony, in the carcass from the market - 31 colonies and in the carcass from the farm - 35 colonies.

The bacterial microflora determined on smears indicates the following data: on the surface of the rabbit carcass from the store and from the farm – 30 colonies, on the carcass from the market 70 colonies, and in depth respectively 10, 30 and 50 colonies.

References

1. **Banu, C.,** Tratat de industrie alimentară, Ed. ASAB, 2010.
2. **Bogdan, A., Ţogoe, Iu., Cîmpeanu Gh.,** Microbiologia alimentelor, Vol.2, Ed. Asclepius, Bucureşti, 2011.
3. **Bogdan, A., Ţogoe, Iu., Cîmpeanu, Gh.,** Microbiologia alimentelor, Vol.1, Ed. Asclepius, Bucureşti, 2011.
4. **Bud, I., Vlădău, V., Petrescu-Mag, V.,** Raising rabbits, Ed. Ceres, Bucharest, 2011.
5. **Carp-Cărare C.,** Microbiologie generală, Ed. Ion Ionescu de la Brad, Iaşi, 2014.
6. **Dan, S., Mihaiu, M., Dalea, I.,** Researches regarding the psychrotrophe microbial load and configuration from meat products, Buletin USAMV-CN, 2006, 64, 251-256.
7. **Dan, V.,** Microbiologia alimentelor, Ed. Alma, Galaţi, 2001.
8. **Darie, N.,** Biochimia alimentară dinamică, Ed. ULB, Sibiu, 2001.
9. **Golban, R.,** Microbiologie alimentară, Curs de prelegeri, UASM, Chişinău: 2015.
10. **Guguianu, E.,** Bacteriologie general, Ed. Jenus, Iaşi, 2002.
11. **Josan, N.,** Microbiologie şi imunologie, UASM, Chişinău, 2002.

12. **Oprean, L.,** Microbiologia produselor alimentare, Vol. 2, Ed. Univ. „Lucian Blaga", Sibiu, 2002.
13. **Răpuntean, Gh., Răpuntean, S.,** Bacteriologie veterinară specială, Ed. Academic Pres, Cluj-Napoca, 2005.
14. **Stănciulescu, M., Sârbulescu, V.,** Animal productions, Ed. Ceres, Bucharest, 2012.
15. **Sunde, M., Norström, M.,** The prevalence of, associations between and conjugal transfer of antibiotic resistance genes in Escherichia coli isolated from Norwegian meat and meat products, Journal of Antimicrobial Chemotherapy, 2006, 58, 4, 741-747.
16. **Taşbac, B.,** Bacteriologie veterinară specială, Ed. Larisa, Câmpulung, 2016.
17. **Taşbac, B.,** Microbiologie generală alimentară, Ed. Larisa, Câmpulung Muscel, 2018.
18. **Taşbac, B., Ţogoe, I.,** Microbiologia alimentelor, Ed. Larisa, Câmpulung Muscel, 2018.
19. **Government Decision no. 696 of 04.08.2010**, regarding the approval of the Technical Regulation "*Meat - raw material. Production, import and marketing*" for the approval of the Requirements for the production, import and placing on the market of meat - raw material.
20. **GOST 26668-85 – 1985,** Food and taste products. Sampling methods for microbiological analysis, Moscow, Iz. Reprint, 2008.

STUDY REGARDING THE ENDOPARASITISM IN GOATS FROM DOLJ COUNTY

MARIN A.M.[1], MORARU M.M.F.[1], POPOVICI D.C.[2], SÎRBU B.A.M.[1], CIREȘAN C.A.[1], PAVLOVIĆ I.[3], ALBU KAYA, M.G.[4], MEDERLE N.[1]

[1]Banat's University of Agricultural Sciences and Veterinary Medicine" King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645, No.119 Calea Aradului, Timisoara, Romania
[2]University Transilvania Brasov, Forestry Faculty
[3]Scientific Veterinary Institute of Serbia, V. Toze 14, 11000 Belgrade, Serbia
[4]Natl Res & Dev Inst Text & Leather, Div Leather & Footwear Res Inst, Dept Collagen, Bucharest 031215, Romania
E-mail: a.marin10@yahoo.com

Summary

The prevalence of gastrointestinal parasites in goats is associated with various environmental and host related factors. The environmental factors include specificity of area, temperature, quantity and quality of pasture, humidity, and grazing behavior of the goat. Moreover, the results of endoparasites on productions is obviously. In this context, the purpose of this study was to identify the presence of endoparasites in a herd of goats in Craiova, Dolj County, using classical coproparasitological methods, macroscopic and microscopic examination of gastrointestinal mass and organs from goats. We identified, morulated stongilid eggs (gastrointestinal nematodes), *Nematodirus spp.* eggs, protozoan oocysts, *Trichocephalus* eggs and oviger proglots (cestodes oncosfers). In conclusion, we recommend a responsible parasitological control, focused on pasture and the correct administration of anthelmintics.
Keywords: goat, endoparasitism, Dolj County.

The prevalence of gastrointestinal parasites in goats is associated with various environmental and host related factors. The environmental factors include temperature, quantity and quality of pasture, humidity and grazing behaviour of the goat. The host species, sex of animal, age, body condition and breed/genotype are considered as risk factors associated with the host. However gastrointestinal parasitic infections development is related to species of the parasite and initial worm population intensity (16, 19).

Intestinal coccidiosis is one of the most important parasitic diseases of small ruminants worldwide. Several *Eimeria spp.* including *E. arloingi* and *E. ninakohlyakimovae* in goats are the most pathogenic. Stress factors such as weaning, inclement weather, dietary changes, traveling and regrouping have important roles in small ruminant coccidiosis (15).

A high prevalence of gastrointestinal (GI) nematodes and coccidial oocysts were reported in countries with tropical and temperate regions such as India, Bangladesh, South Africa, Sri Lanka, Italy and Mongolia with the prevalence rate ranging from 20–96% (9).

The cestodosis of small ruminants is caused by species belonging to the *Moniezia, Thysaniezia, Avitellina, Stilesia, Thysanosoma* genera. The infection with *Moniezia spp.* is more frequent in lambs and goat kids, in the first year of their life and, more rarely, is encountered in young and adult animals (13). The prevalence of cestodes in small ruminants is variable. In some cases, *Moniezia spp.* registered more values in sheep (69.0%), comparatively with goats (55.0%) (11).

Goats are very often affected by *Haemonchus contortus* throughout the world. *H. contortus* is the most pathogenic of the common nematodes, particularly in young animals and its blood-feeding behaviour results in severe anemia and weight loss, with death eventually occurring in cases of heavy infection (21).

In this context, the purpose of this study was to identify and to evaluate the presence of endoparasites in a herd of goats in Craiova, Dolj County, using classical coproparasitological methods, macroscopic and microscopic examination of gastrointestinal mass and organs from goats.

Materials and methods

The study was conducted on a private property with a population of 27 goats of the Carpathian race, with age between two-five years. Goats are maintained on dry pastures, but also wet, with / without swampy land (Fig. 1).

Fig. 1. The herd of goats from Craiova

Between 50 and 150 g of freshly discarded feces were collected or directly from the rectum of the goats under study. The samples were collected in coprocultures and refrigerated until processing.

Gastrointestinal masses and organs (lung, liver, CNS) were also harvested.

The samples were transported and examined in the Parasitology and Parasitic Diseases Clinics of the Faculty of Veterinary Medicine, Timișoara and were processed by the following methods:

- Qualitative method - identification of the parasitic load of the whole flock with light eggs of nematodes, cestodes, protozoan oocysts.
- Polyvalent method (of successive washes) - identification of the presence of trematode eggs.
- Larvoscopic method - highlighting parasitism with pulmonary nematodes.
- Necropsy examination - in sacrificed individuals, according to the technical instructions of necropsy (Fig. 2) (5, 6).

Fig. 2. The action of skinning the animal

Results and discussions

The results of the coprological examination performed by the flotation method revealed the presence of the following parasitic elements: morulated stongilid eggs (gastrointestinal nematodes, *Nematodirus spp.*), oocysts of protozoa, eggs of *Trichocephalus* and oviger proglots (cestodes oncosfers) (Fig. 3 - Fig. 7).

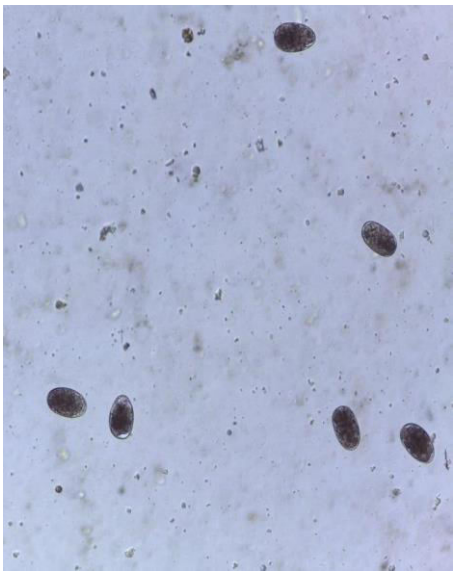


Fig. 3. Nematodes eggs

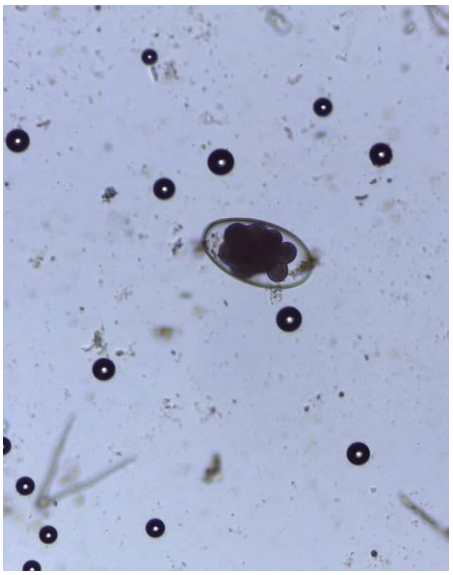


Fig. 4. *Nematodirus spp*. egg

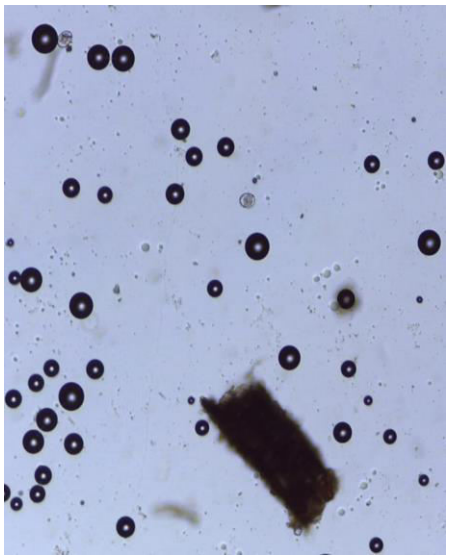


Fig. 5. Oocyst of protozoa

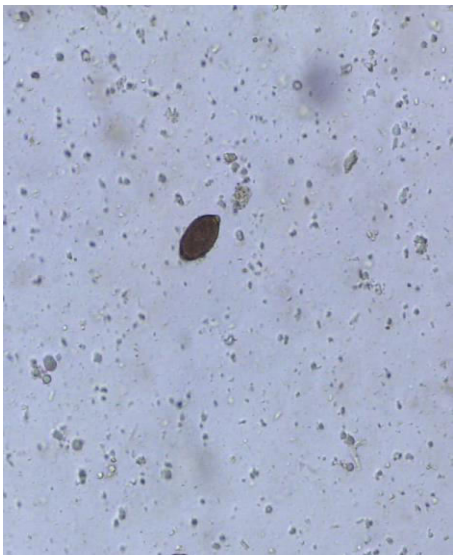


Fig. 6. *Trichocephalus* egg

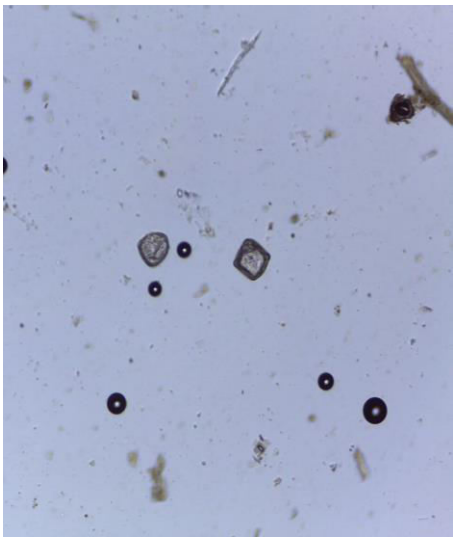


Fig. 7. Oviger proglots (cestodes oncosfers)

Fig. 8. *Dicrocelium lanceolatum* egg

The results of the coprological examination performed by the sedimentation method, respectively, revealed the absence of parasitic elements belonging to the genera *Fasciola*, *Paramfistomum spp*. The only parasitic elements identified following this coprological method were the heavy eggs of *Dicrocelium lanceolatum* (Fig. 8).

The results of the coprological examination performed by the Baermann method revealed the absence of parasitic elements belonging to the genera *Dictiocaulus* and *Protostongilus spp*.

The results of the necropsy examination of the gastrointestinal mass revealed the presence of different types of gastrointestinal nematodes, of which the high prevalence was *Haemonchus* spp. in abomasum (Fig. 9, 10).

Only the trematodes *Dicrocelium lanceolatum* were present in the liver of the examined goats (Fig. 11).

Fig. 9. Necropsy examination

Fig. 10. *Haemonchus contortus*

Fig. 11. *Dicrocelium lanceolatum*

The coprological examinations performed on a number of 27 adult goats revealed the following results:

- presence of *Eimeria* oocysts at 10/27 samples collected (37%),
- presence of cestode oncospheres in 15/27 samples collected (55%),
- presence of gastrointestinal nematodes eggs 25/27 samples collected (93%),
- presence of *Trichocephalus* eggs at 7/27 samples collected (26%),

- the presence of eggs *Dicrocelium lanceolatum* at 4/27 samples collected (15%).

Necropsy examinations performed on five adult goats had the following results:

- identification of *D. lanceolatum* in the liver of for goats;
- identification of cestodes in the intestine of the five goats;
- identification of gastrointestinal nematodes in all examined goats.

Aspects related to the distribution and spread of internal parasites in goats, specificity of areas, presence or absence of certain factors that determine different manifestations of endoparasitosis, host-parasite relationship, links between biotic and abiotic components involved in biological cycles, survival strategies of parasites, and the results of endoparasitism on productions are subjects of study approached in numerous scientific papers (1, 2, 3, 4, 7, 17).

The results of a study conducted in India join those obtained in this study, climate (rainy season), grazing conditions, moisture maintained on plots being the factors that obviously influenced the high prevalence of gastrointestinal nematodes. Cestodes and protozoa were located immediately after gastrointestinal strongyls, which correlates with similar percentages of the prevalence of these endoparasites identified in goats in Craiova (8).

A study (2021) conducted in southern Africa on a herd of 765 goats reveals a prevalence of gastrointestinal parasites of 94.7%. In the analyzed coprological samples were identified nematodes (64.7%), coccidia (25.8%) and cestodes (4.2%) (16).

In the same year (2021), a study conducted in Thailand by Junsiri et al. (14) reports that the prevalence of gastrointestinal parasites in goats is 86.54%. In the present study, *Strongyloides spp.* (57.77%), *Paramphistomum cervi* (33.43%), *Moniezia spp.* (29.91%) and *Eimeria spp*. (1.75%) were identified (1.75%) (14).

In our country, Sîrbu et al. (20), revealed the importance of gastrointestinal strongyl parasitism in small ruminants, their prevalence being 92.6%, respectively followed by protozoan infestation (24.1%).

Eimeriasis is one of the major parasitic diseases diagnosed in the world in small ruminants, with a strong impact on youth. Among the species involved in the etiology of the disease, *E. arloingi, E. ninakohlyakimovae* and *E. christenseni* are considered by Khodakaram-Tafti et al. (15) and Mohamaden et al. (18) to be the most pathogenic (18).

Hotea et al. (10) draw attention to another protozoan important for the health of small ruminants. It is about *Toxoplasma spp.* identified by serological examinations (ELISA) in 75 percent of the goats studied, breed, age and presence of cats, the definitive host, representing the main factors involved in the epidemiology of protozoan (10).

Correlating the results obtained with the concrete epidemiological situation in the investigated area (Craiova, Dolj County), we can draw some measures that we recommend to be applied in order to reduce the existing parasitic infrapopulation

(gastrointestinal nematodes, trematodes, cestodes, protozoa) or to maintain it to an unfavorable degree from an economic perspective. Thus, the application of a correct plan of pasture management, reconsideration of therapeutic protocol, administration of good quality food, avoidance of areas on pasture with high humidity or areas conducive to the development of intermediate hosts, collection of feces from infested animals, followed by biosterilization there are some important aspects whose responsible application can serve the purpose.

In the same way, authors from the country and abroad recommend a responsible parasitological control, focused on pasture and the correct administration of anthelmintics (12, 18, 20).

Conclusions

In a herd of goats from Craiova (Dolj County) we identified, following coproparasitological examinations, morulated stongilid eggs (gastrointestinal nematodes), *Nematodirus spp.* eggs, protozoan oocysts, *Trichocephalus* eggs and oviger proglots (cestodes oncosfers).

The necropsy examination of the gastrointestinal mass allowed us to highlight the parasitism with gastrointestinal nematodes.

Macroscopic examination of the organs revealed the presence of *Dicrocelium lanceolatum* in the liver.

Acknowledgments

This scientific paper was carried out in the Laboratory of Parasitology and Parasitic Disease at the Faculty of Veterinary Medicine Timisoara, Laboratory within the Animal Hygiene and Pathology Research Center/ BUASVM Timisoara.

References

1. **Constantin, N., Darabus, Gh.,** Tratat de Medicină Veterinară, Ed. Risoprint, Cluj Napoca, 2014.
2. **Dărăbuş, Gh.,** Controlul parazitologic în helmintozele ierbivorelor - partea I, Revista Română Medicină Veterinară, 2003, 13, 2, 70-81.
3. **Dărăbuş, Gh.,** Controlul parazitologic în helmintozele ierbivorelor – partea a II-a, Revista Română Medicină Veterinară, 2004, 14, 1, 58-66.
4. **Dărăbuş, Gh., Oprescu, I., Morariu, S. Mederle, N.,** Ediție revizuită, Parazitologie și Boli Parazitare, Ed. Mirton, Timişoara, 2019.
5. **Dărăbuş, Gh., Oprescu, I., Morariu, S. Mederle, N., Ilie, M.,** Ghid practic în bolile parazitare, vol. I, Ed. Agroprint, Timişoara, 2013.
6. **Dărăbuş, Gh., Oprescu, I., Morariu, S. Mederle, N., Ilie, M.,** Ghid practic în bolile parazitare, vol. II, Ed. Agroprint, Timişoara, 2013.

7. **Dărăbuş, Gh.,** Principii generale de control al helmintozelor la ovine, Revista Română Medicină Veterinară, 1998, 4, 17-29.
8. **Das, M., Laha, R., Goswami, A., Sen, A.,** Gastrointestinal parasitism of goats in hilly region of Meghalaya, India, Veterinary World, 2017, 10, 1, 81-85.
9. **Gebeyehu, E.B., Seo, M.G., Jung, B. Y., Byun, J.W., Oem, J.G., Kim, H.Y., Kwak, D.,** Prevalence of gastrointestinal parasites in Korean Native goats (Capra Hircus Aegagrus), Journal of Animal & Plant Sciences, 2013, 23, 4, 986-989.
10. **Hotea, I., Herman, V., Tîrziu, E., Colibar, O., Brudiu, I., Sîrbu, C., Dărăbuș, Gh.,** Seroprevalence and risk factors for *Toxoplasma gondii* infection in sheep and goats from Romania, Parasitologia, 2021, 1, 2, 36-44.
11. **Iacob, O.C., El-Deeb, W.M., Paşca, S.A., Turtoi, A.I.,** Uncommon co-infection due to *Monezia expansa* and *Monezia benedeni* in young goats from Romania: morphological and histopathological analysis, Annals of Parasitology, 2020, 66, 4, 501-507.
12. **Inani, R.N.R., Yusof, A.M.,** Seasonal prevalence of gastrointestinal parasitic infection in goats in a commercial farm Kuantan, Malaysia, Asian Journal of Agriculture and Biology, 2018, 6, 4, 455-460.
13. **Income, N., Tongshoob, J., Taksinoros, S., Adisakwattana, P., Rotejanaprasert, C., Maneekan, P., Kosoltanapiwat, N.,** Helminth Infections in Cattle and Goats in Kanchanaburi, Thailand, with Focus on Strongyle Nematode Infections, Veterinary Sciences, 2022, 8, 12, 324.
14. **Junsiri, W., Tapo, P., Chawengkirttikul, R., Watthanadirek, A., Poolsawat, N., Minsakorn, S., Anuracpreeda, P.,** The occurrence of gastrointestinal parasitic infections of goats in Ratchaburi, Thailand, Thai Journal of Veterinary Medicine, 2021, 51, 1, 151-160.
15. **Khodakaram-Tafti, A., Hashemnia, M.,** An overview of intestinal coccidiosis in sheep and goats, Revue de Medecine Veterinaire, 2017, 168, 1-3, 9-20.
16. **Matsepe, L.G., Molapo, S., Phalatsi, M., Phororo, M.,** Prevalence and fecal egg load of gastrointestinal parasites of Angora goats in four agro-ecological zones in Lesotho, Veterinary World, 2021, 14, 2, 339-346.
17. **Mitrea, I.L.,** Bolile parazitare la animale, Ed. Ceres, Bucureşti, 2002.
18. **Mohamaden, W.I., Sallam, N.H., Abouelhassan, E.M.,** Prevalenceof *Eimeria spp.* among sheep and goats in Suez Governorate, Egypt, International Journal of Veterinary Science and Medicine, 2018, 6, 1, 65-72.
19. **Pilarczyk, B., Tomza-Marciniak, A., Pilarczyk, R., Bombik, E., Seremak, B., Udala, J., Sadowska, N.,** A Comparison of the Prevalence of the Parasites of the Digestive Tract in Goats from Organic and Conventional Farms, Animals, 2021, 11, 9, 2581.
20. **Sîrbu, C.B., Imre, K., Dărăbuș, Gh., Suici, T., Mates, B., Morariu, S.,** Prevalence of gastrointestinal parasitic infections in cattle and sheep in two

regions of Romania, Turkish Journal of Veterinary and Animal Sciences, 2020, 44, 581-587.

21. **Vongnady, K., Rucksaken, R., Mangkit, B.,** Morphological identification of adult male Haemonchus species in goats from Thailand and Lao PDR, Tropical Biomedicine, 2020, 37,3, 691-712.

BIOFILMS IN THE FOOD INDUSTRY: A FOCUS ON THE METHODS USED FOR DETECTION AND STUDY OF MICROBIAL BIOFILMS

MOZA A.C., TÎRZIU E.

Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645,
Calea Aradului No. 119, Timisoara, Romania
E-mail: alex.moza@usab-tm.ro

Summary

Biofilms represent microbial communities that live embedded in a self-secreted extracellular polymeric substance which grants them protection and vital nutrients, and are attached to a biotic or abiotic surface. The biofilm is a survival strategy implemented by microbes to survive in difficult environmental conditions. Their presence on the food-industry surfaces poses a great challenge for these industries, increasing food cross-contamination which will then lead to economic losses through food decay, and damaged processing equipment, public health issues, and eventually outbreaks. Due to the biofilms raising negative potential, many interdisciplinary researchers were determined to study biofilm characteristics in order to improve antibiofilm strategies and prevention methods. The final result is a multitude of knowledge and inventive technologies. In this article, we will review some of the most frequently used model systems for detection and study of biofilms in the food industry, ranging from traditional methods, to cutting-edge assays. Furthermore, this review can assist novice researchers in choosing the appropriate study methods.

Keywords: biofilms, biofilm study methods, food industry, food safety.

In the past, it was believed that microorganisms existed in the form of planktonic or free floating cells. The last decades were revolutionary for the microbiology universe, when Costerton discovered the aggregates of microbes, today known as „biofilms" (13).

Microbial biofilms can be defined as heterogeneous or homogeneous bacterial communities surrounded by a sticky self-secreted matrix made of exopolymeric substances (EPS), that are present on both, biotic and abiotic wet surfaces (26). The typical biofilm architecture is comprised of bacterial cells, both living and dead, the extracellular polymeric substances and other components and metabolites secreted by the cells (18).

Biofilms provide multiple advantages for the embedded microorganisms, such as protection from harsh environmental conditions including desiccation, radiation, predation, tolerance to antimicrobial compounds, increased resistance to host immune response, and retaining vital nutrients for the microorganisms (25, 48). Within a biofilm, communication between cells is realised through small molecules in order to coordinate the activities critical to their survival, which can also influence the biochemical composition and structure of the microbial aggregates.

Moreover, the close proximity of cells provides the perfect environment for gene transfer, which contributes, as well, to the development of antimicrobial resistance of microbial pathogens (10, 13, 38). It was suggested, that the adaptability of the matrix represents a key component of biofilms remanence in hostile environments, due to the superficial exposure of cells to antimicrobial agents (44).

It is well known that both, bacteria and fungi are capable of forming such aggregates on a large variety of surfaces (13). Despite the fact that in some industries biofilms have beneficial effects, we must not forget that there are also biofilm-forming pathogens which represent a major health hazard being the cause of 60-80% of infections and presenting a true challenge when it comes to diagnosis and treatment (20).

Regarding the food industry, biofilms are a major public health problem as well, because they allow bacteria to attach to various food processing equipment surfaces, such as stainless steel, plastic, rubber, polypropylene, glass, and even contaminate the final food products. The adhesion of bacteria occurs within just a few minutes, followed by development of mature biofilms in a matter of hours or days (47). Hence, biofilms can cause economical and productivity losses, equipment deterioration, product recall, food spoilage, and potential food poisoning, or even outbreaks (9). When biofilms combine with meat exudate, it results in slime formation which is a key quality defect that leads to consumer rejection of meat (42).

Across a variety of food industries, such as dairy processing, seafood processing, brewing, and meat and poultry processing, biofilms have become more resisting to the disinfection procedures (12).

Nevertheless, several authors consider them a real threat, and have shown the impact of these microbial communities in the food industry, due to cross-contamination with various pathogens, including *Listeria monocytogenes*, *Campylobacter jejuni*, *Bacillus cereus*, *Salmonella spp.*, *Yersinia enterocolitica*, *Staphylococcus spp.* and *Escherichia coli* O157:H7 (4, 11, 49). *Pseudomonas fragi* is worldwide the most often encountered spoilation microorganism on stored fresh meat including pork, lamb, chicken, fish and beef, and is able to form biofilms in conditions of refrigeration that are used in the meat industry (16, 17, 42).

At the moment, the methods used to study biofilm are not standardized, and aim to analyse formation and development of biofilm. Different techniques have been utilized to evaluate biofilms from a quantitative and qualitative point of view, and are individually useful for observing on particular aspect of the bacterial community (12). It is imperative to study microbial biofilms in situ, or by using experimental model systems with industrial applicability, due to the metabolism and genes expression which are subject to change depending on the environment conditions in which they grow (43, 46). Regarding the food industry, model systems should be designed so that they closely represent the spoilage conditions.

Continuous research over time has allowed some techniques to be developed or adapted to evaluate biofilms. Therefore, we should choose the

appropriate methods of studying a biofilm, according to the objectives of our research.

In order to aid the development of more efficient ways to control harmful biofilms, a deeper knowledge on them, as a whole community of cells and at a solitary cell level, is imperative.

In this article, we will review some of the most frequently used model systems that were used by researchers to detect and study biofilms in food industry, ranging from traditional methods, to cutting-edge assays.

Methods for biofilm identification and visualisation in situ

There are several approaches when it comes to classifying the biofilm study techniques. Consequently, the most fundamental classification of methods would be direct or indirect. Another way is to classify the techniques as rapid hygienic control tests, and microscopic, biomolecular, EPS evaluation methods. One of the most relevant methodology for biofilms study is presented in a recent publication, and it classifies the technology of the researching methods in physics, chemistry and lastly physico-chemistry; this authors also suggest the combined use of three major procedures for examining biofilms: (i) different microscopical methods; (ii) detailed analysis of data while processing microscopic image; (iii) combined chemical analysis with atomic force microscopy (AFM) (21). By doing so, not only that we obtain detailed surface images of the biofilm, but we can also observe the relations between the matrix, inner interactions, pH, surfaces for attachment and other factors (12).

Biofilms can be comprised of one or multiple species of bacteria, having a rich structural complexity and heterogeneity, therefore the techniques should be adapted to the research objectives.

1.Direct microscopic methods

Techniques such as light microscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM) can be used to observe 3D structures, complexity and dynamics of biofilms (33).

Light microscopy

Light microscopy is a fast, simple, cheap and direct method to examine the morphology of biofilm-forming microorganisms which adhered to surfaces. Moreover, it can be used to evaluate the amount of cells attached on the surface (rare, abundant, absent, existing, and so on) (22).

This method of examination requires surfaces with certain characteristics, such as transparent, clear and even surface, in order to prevent the adhered

microorganisms from creating 3D biofilms. To better highlight the cells, epifluorescence and fluorescent dyes can be used. Through light microscopy, the morphology of both sessile and planktonic forms of the microbes can be compared. Pathogens such as *Escherichia coli*, *Staphylococcus epidermidis*, *Candida albicans*, and *Pseudomonas spp*. were studied with light microscopy while they were attached to polystyrene Petri dishes, glass coverslips, and acrylic sheets of polymethacrylate films (22, 47).

Scanning electron microscopy (SEM)

Scanning electron microscopy is a very useful technique for studying biofilms, providing high resolution images of surface topography, EPS structure, data regarding the size, shape or localisation of single bacteria in the biofilm due to its great magnification range (10-500,000 times) (1). Overall, SEM is a highly valuable technique for analysing the microbial communities adhered to surfaces, and better understanding their formation and resistance mechanisms (12).

The way that scanning electron microscope works is comparable to conventional fluorescent microscope, the difference lays between the beam of electrons used by SEM versus the beam of photons used by the latter one (44).

The electron beam passes through electromagnetic lenses, reaches the sample from where the electron gets absorbed by the surface of probes and the secondary electron is ejected, or is scattered creating so called back-scattered electrons. These electrons are then captured by special sensors in order to create a digital image (1).

Images obtained from the secondary electron represent the main SEM imaging technique, meanwhile back-scattered electron will generate low resolution images, indicating chemical variations and their locations (44).

Unfortunately, SEM method cannot be applied on living cells, due to conditions and preparations required for the examination of biofilm samples. Thus, testing is sustained under vacuum condition, and preparation of samples require fixation, dehydration, and in order to obtain 3D images of cells, these are being coated with conductive metals (gold, gold palladium or platinum), which can destroy the sample structure (22). Taking into consideration that biofilms contain over 95% water, the process of dehydration will unavoidably affect both structure and size of samples (6).

Confocal laser scanning microscopy (CLSM)

CLSM is a specialized type of microscopy that offers sharp, high-quality, and three-dimensional (3D) images of biofilms (28). It uses an optical microscope implemented with a laser beam, and is very effective in studying thick biofilms and their layers which contain biofilm-embedded microorganisms. The morphology and

physiology can be investigated with CLSM in 3D regarding biofilms that were formed on flow cells, thanks to the transparent surface (36).

Biofilm samples require to be fluorescent in order to be observed with confocal microscopy. The fluorescent light is captured by the microscopes optics and transformed into electrical signal by a photodiode, finally being computer-processed (12). The microscope focuses on a small area of the sample, and while scanning across its thickness it creates many two-dimensional (2D) images at various heights, images that will be assembled in order to create a 3D image. Multiple fluorescent markers can be viewed simultaneously due to the fact that the confocal microscope can use multiple lasers (44).

Fluorescence can be acquired by staining the samples with fluorescence dyes. EPS can be examined by using stains such as lectins, meanwhile extracellular DNA (eDNA) can be visualised using certain fluorophores (22, 36).

A fluorescent biofilm can also be obtained by tagging of certain microbial gene, by gene cassette, which is encoding the green fluorescent protein (GFP). This protein will be expressed by biofilm-forming microbes, and can be used to monitor the activity and growth phase of bacteria within biofilm, due to the fact that the expression cassette is active only in growing cells (36).

Given the structural complexity of the heterogenous microbial biofilm matrix, no fluorescence staining method is yet available that could allow a full visualization of if, therefore, each matrix element has to be individually stained. Moreover, there is a lack in polysaccharides general staining due to differences between the chemical structure of polysaccharides elaborated by Gram positive and Gram negative bacteria (34).

Fluorescent in situ hybridization (FISH)

Fluorescent in situ hybridization method can be used to study microbes that are present in a diversified biofilm and spatial structure of mixed biofilms (8, 22).

This method involves fluorescently labelled DNA probes that are designed to hybridize rRNA, which is abundant in viable microorganisms, in the microbial cells that were fixed and permeabilized in order to permit the entry of probe (8). The probes are combined with fluorescent dye (Rhodamine B or Fluorescein isothiocyanate) or an enzyme called horseradish peroxidase. The latter one has the advantage of not killing the bacterial cells from the biofilm. As in the case of CLSM, by using tagged cells to encode the GFP the technique will not require a further fixation or staining (36).

The advantages for this method are the fast and precise detection of specific DNA, it can be executed even in cells that are not actively dividing and the growth activity of the microorganism can be determined. Unfortunately, however, it has also drawbacks such as low probe permeability, insufficient probe hybridization capability, and it is limited in simultaneous detection of multiple microorganisms (8).

In the case of multispecies biofilms, FISH technique can be combined with CLSM in order to identify and visualize different microbial species more accurately (30).

2.Colorimetric methods

Through colorimetric methods, the biofilm matrix, biofilm-embedded cells and cell components can be stained to evaluate the biofilm formation capacity and metabolic status of the microorganisms within a biofilm. These methods are widely used by researchers to highlight the microbial biofilms that were grown in microtiter plate wells.

One of the most frequently used staining method is crystal violet, and is used to quantify biofilm biomass. It stains both biofilm matrix and the biofilm-embedded microbes (15). This method has the disadvantage of staining both, living and dead cells, with the condition of cell integrity. Thus, it cannot be used to quantify the living population of bacteria (12). Biofilm biomass can also be observed with safranin staining. This, however, has a lower sensitivity in detecting smaller amounts of biofilm, due to lower optical densities compared to crystal violet (29).

ATP bioluminescence is a staining method that reveals the microbial cell's metabolic condition. After the cell's death, the staining scales down to undetectable values. By comparing results of ATP bioluminescence technique with those of crystal violet staining, it was noticed that despite a radical drop in living cell numbers in a biofilm after applying a disinfectant treatment, crystal violet dye could still stain a high number of intact cells or cellular debris. Due to these results, it is believed that crystal violet staining is not a trustworthy method to monitor disinfection quality on biofilms (32).

Fluorescein diacetate is yet another staining method for highlighting living cells, using a fluorescent component that is metabolised in fluorescein within living cells. Afterwards, the cell viability can be quantified through spectrophotometry, as dead cells cannot metabolise the staining substance (12).

LIVE/DEAD staining is used to evaluate examine live and dead bacteria using fluorescent microscopy. This assay requires two nucleic acid binding dyes, the green fluorescent (Syto9) and red-fluorescent propidium-iodine. The first one passes through all cell membranes in order to bind with the DNA of both Gram positive and Gram negative bacteria, while the second stain is able to cross only damaged bacterial cells. Therefore, living bacteria will present a green fluorescence, and dead ones will be stained with an orange/red fluorescence (12, 30).

LIVE/DEAD staining can be combined with CLSM, fluorescence microscopy, fluorometry flow, and cytometry. However, it has no practice efficiency in directly staining biofilms that are grown on surfaces because the biofilm matrix and slime will disrupt the stain (12).

Another way to highlight and differentiate cells from organic matter is through different fluorescent staining. These techniques can be applied to stain food-

contact surfaces and microorganisms on them, in order to evaluate surface hygiene. Therefore, staining techniques comprised by combinations of DAPI (4', 6-diamidino-2-fenilindol) and Rhodamine B, or DAPI and fluorescein, or acridine orange are useful (12). In a study, the efficiency of various dyeing methods was compared, after which Rhodamine B combined with DAPI obtained the best results in quantitatively determining *L. monocytogenes* under epifluorescence microscopy (41). Regarding biofilm-forming pathogens, DAPI staining can be used to examine cell viability during initial attachment of microorganisms to surfaces, while they are still in planktonic form (2). As mentioned before, DAPI/Rhodamine B combined are useful in quantifying the organic matter of biofilms and biofilm-embedded cells, separately. In another research, Almeida et al. (2) used DAPI staining combined with peptide nucleic acid FISH (PNA FISH) in order to study the initial adhesion, followed by formation of biofilm of three species of bacteria: *Escherichia coli*, *Salmonella enterica* and *Listeria monocytogenes* (12).

3.Microbiological methods and biofilm growing devices

Determination of viable cell numbers by plate count (CFU/ml)

CFU/ml assay is widely used to determine the viable cells within a biofilm by colony forming units on agar medium. This method is very simple to execute in any microbiology laboratory, being based on the universal serial dilution used to quantify cells. In food industry, samples with biofilms from different surfaces are acquired through swab or sonication and finally discharged on agar plates. The culture media can be either non-specific, or specific for a certain species. Unfortunately, this assay has major disadvantages and limitations, such as (i) the cells that will detach and compose the sample may not contain the same properties as the initial biofilm population, and (ii) even though some subpopulations of biofilm cells are viable, they may be non-culturable (VBNC), thus not being detected by CFU assay (24). To overcome this, a combination of flow cytometry and fluorophores was proposed as an alternative to determination of total viable cells from biofilms (14).

Congo Red Agar (CRA)

This is a qualitative and indirect method for distinguishing biofilm-forming microorganism, which is based on colour change of colonies that are growing on Congo red agar medium (22). The agar plates are prepared by adding 0.8 g of Congo red and 36 g of saccharose to 1 L of blood agar. Afterwards, the inoculated plates will pass through 24 hours of incubation at 37°C (7). Biofilm-producing bacteria will form crusty black colonies, with dry filamentous appearance, whereas non-biofilm producing microorganism will create pink colonies. Potential biofilm producers will form on CRA medium pink colonies with dark centres (5).

Microtiter plate (MtP)

At present, there is a large variety of commercially available study models for biofilms, one of the mostly used in research being the microtiter plate system (MtP). MtP is a quantitative technique, which uses plates that come with 12, 24 or 96 well-plates in which cultured biofilms will form on the microtiter plate surfaces, or onto a specific surface that is placed inside the microtiter plate wells (13, 23). Biofilm production is determined by microplate reader (22).

MtP is classified as a static study model, due to its limited nutrient supply. There will be no renewal of the culture medium during the incubation period, and the conditions from within the wells will suffer a continuous change due to nutrients depletion, accumulation of metabolite and signalling molecules (13).

After the planktonic cells will be discharged by two successive washes with phosphate-buffered saline (PBS), the sessile microbes within the biofilm that is formed on the inside of wells surface, will be stained with safranine in order to be measured by spectrophotometry microplate reader (39).

MtP is a very reasonable assay considering aspects, such as the possibility of executing a substantial number of simultaneous experiments, requiring small quantities of reagents, no need for advanced equipment except from the plate reader, and the total low-cost. These plate-based models are also being used to measure de anti-biofilm actions of different compounds, antimicrobial and disinfectant substances (13, 37).

Besides MtP being a static model of study, another drawback could be that the biofilm quantification methods are conducted with indirect measurements, including crystal violet dye for highlighting the biofilm, and XTT reduction or resazurin methods to evaluate cell viability within biofilm (13, 31). Moreover, weakly attached biofilm can be detached during washing steps and not measured correctly, biofilm mass can be modified by the sedimentation of cells to the base of the wells and the assay is not useful in studying early stages in biofilm formation (8).

In order to overcome the limitations of classical microtiter plate assay, biofilm growing devices such as the Calgary plate and the Biofilm Ring Test can be used (13).

The Calgary biofilm device comprises of a coverlid containing plastic polycarbonate pegs, which will fit into the wells of 96-well microtiter plates that are containing the growth medium and the bacterial inoculum. The plastic pegs act as a surface on which the sessile microorganisms will attach and start forming a biofilm. Therefore, the sedimentation of cells will not modify the biofilm formed on the pegs.

In order to quantify the biofilm biomass, sonication is used to remove cells from the plastic pegs. This technique, however, does not guarantee the detachment of all the sessile cells, usually only a portion of 5-90% of the community being mobilised. In addition, the detached population of cells may not represent the exact physiology of the original sessile microorganisms, due to the fact that adhesion and

detachment properties on surfaces, can vary from a population of cells, to another (8, 19).

Since the coverlids with grown biofilms can be easily moved to another microtiter plates containing fresh culture media, the study model becomes dynamic and the environment in which the biofilms grow will suffer less from metabolites and other regulating molecules. Moreover, biofilm minimal inhibitory concentration of antimicrobials and disinfectants can be easily studied with Calgary device (13, 23).

The Biofilm Ring assay is used to study early stages of biofilm formation, judged by the capacity of microorganisms to immobilize paramagnetic microbeads with the biofilm EPS, at the surface. The experiment is conducted in microtiter plate wells preloaded with bacterial suspension and microbeads. The main principle is simple, the more biofilm is produced, the less the microbeads move under a magnetic field. The Biofilm Ring device offers valuable information about how different components, both produce by the biofilm or to be tested, can influence the adhesion and growth of biofilm, also to compare the biofilm-forming mechanism of different pathogens (8, 13).

Tube method (TM)

Tube method is a simple, indirect and qualitative test for detection of visible biofilms. It uses test tubes containing tryptic soy broth which will be inoculated with microbial isolates and incubated at 37°C for 24 hours. During the incubation period, some of the sessile microbial cells with adhere to the walls of test tubes. Afterwards, the planktonic forms will be washed away two consecutive times with PBS and the biofilms will be stained for 1 hour with safranine. The staining is followed up by another two consecutive rinsing with PBS, and air drying, after which the existing biofilms will be visible on the walls and at the bottom of test tubes (22).

4.Genetic Assays

Polymerase chain reaction (PCR) and Real-Time Quantitative Reverse Transcription PCR (qRT-PCR)

Polymerase chain reaction has been frequently used as method of diagnosis. Through this method, specific genes related to an individual microbial species can be accurately identified, thus, being efficient in studying multispecies biofilms. However, because the amplified DNA can be represented by both, dead and living cells, and extracellular DNA (eDNA), PCR is not a recommended quantitative assay and cannot be used in quantitative research (30). In order to avoid the quantification of non-living cell´s DNA, samples can be treated with propidium monoazide (PMA) before extracting the DNA (3). The PMA molecules will bond with eDNA and the DNA of dead cells, modifying their structure. As a result, during the extraction process, the modified DNA will be lost and not amplified during PCR (27).

As a solution to the PCR limitations, Real-Time Quantitative Reverse Transcription PCR (qRT-PCR) was approved in examining microbial biofilm. While traditional PCR amplifies all of the DNA present in the biofilm sample, the latter method is able to identify and quantify individual microorganisms in the biofilm. With qRT-PCR the mRNA of microbes was proposed as an indicator that would represent cell viability (30, 35, 40, 45).

This assay is expensive, requires meticulous sample preparation and skilled staff, but is a fast and accurate method.

Conclusions

Over the last decades, biofilms came to pose a major concern for industries, such as the food industry. These microbial communities are, even today, an intriguing topic due to food cross-contamination, food poisoning outbreaks and presenting an extraordinary tolerance towards antibiotics. It is well known that a considerable number of food-borne pathogens are capable of biofilm-production, therefore their presence on food-industry surfaces must be prevented. The study of biofilms implies techniques ranging from traditional methods, to cutting-edge assays, and it is imperative for researchers to enrich their knowledge in order to accurately choose their study models depending on the objectives of research. This review provides new researchers with a quick review that aims biofilm detection and study methods.

References

1. **Adetunji, V., Odetokun, I.A.,** Assessment of biofilm in *E. coli* O157:H7 and *Salmonella* strains: Influence of cultural conditions, American Journal of Food Technology, 2012 ,7, 582-595.
2. **Almeida, C., Azevedo, N.F., Santos, S., Keevil, C.W., Vieira, M.J.,** Discriminating multi-species populations in biofilms with peptide nucleic acid fluorescence in situ hybridization (PNA FISH), PLoS One, 2011, 6, 3.
3. **Alvarez, G., González, M., Isabal, S., Blanc, V., León, R.,** Method to quantify live and dead cells in multi-species oral biofilm by real-time PCR with propidium monoazide, AMB Express, 2013, 3, 1, 1.
4. **Anand, S., Singh, D., Avadhanula, M., Marka, S.,** Development and Control of Bacterial Biofilms on Dairy Processing Membranes, Comprehensive reviews in Food Science and Food Safety, 2014, 13, 1, 18-33.
5. **Arciola, C.R., Campoccia, D., Gamberini, S., Baldassarri, L., Montanaro, L.,** Prevalence of cna, fnbA and fnbB adhesin genes among *Staphylococcus aureus* isolates from orthopedic infections associated to different types of implant, FEMS Microbiology Letters, 2005, 24, 1, 81-86.
6. **Asahi, Y., Miura, J., Tsuda, T., Kuwabata, S., Tsunashima, K., Noiri, Y., Sakata, T., Ebisu, S., Hayashi, M.,** Simple observation of *Streptococcus*

mutans biofilm by scanning electron microscopy using ionic liquids, AMB Express, 2015, 5, 1, 6.

7. **Avila-Novoa, M-G., Iñíguez-Moreno, M., Solís-Velázquez, O-A., González-Gómez, J-P., Guerrero-Medina, P-J., Gutiérrez-Lomelí, M.,** Biofilm Formation by *Staphylococcus aureus* isolated from food contact surfaces in the dairy industry of Jalisco, Mexico, Journal of Food Quality, 2018, 8.
8. **Azeredo, J., Azevedo, N.F., Briandet, R., Cerca, N., Coenye, T., Costa, A. R., Desvaux, M., Di Bonaventura, G., Hébraud, M., Jaglic, Z., Kačániová, M., Knøchel, S., Lourenço, A., Mergulhão, F., Meyer, R.L., Nychas, G., Simões, M., Tresse, O., Sternberg, C.,** Critical review on biofilm methods, Critical Reviews in Microbiology, 2017, 43, 3, 313-351.
9. **Bixler, G.D., Bhushan, B.,** Biofouling: lessons from nature, Philosophical transactions. Series A, Mathematical, Physical, and Engineering Sciences, 2012, 370, 1967, 2381-2417.
10. **Bucur, I., Popa, V., Cătana, N.,** Research regarding the resistance phenotypes in staphylococci isolated from bovine mastitis, Scientifical Papers: Veterinary Medicine Timisoara, 2015, 48, 2, 36-39.
11. **Bucur, I., Cătana, N., Necşulescu, M., Imre, K., Herman, V., Nichita, I., Tîrziu, E.,** Genotypic differentiation of *S. intermedius* and *S. pseudintermedius* strains isolated from dogs and cats, Revista Română de Medicină Veterinară, 2020, 30, 3, 81-84.
12. **Carrascosa, C., Raheem, D., Ramos, F., Saraiva, A., Raposo, A.,** Microbial Biofilms in the Food Industry - A Comprehensive Review, International Journal of Environmental Research and Public Health, 2021, 18, 4.
13. **Castelo-Branco, D., Amando, B.R., Ocadaque, C.J., Aguiar, L., Paiva, D., Diógenes, E.M., Guedes, G., Costa, C.L., Santos-Filho, A., Andrade, A., Cordeiro, R.A., Rocha, M., Sidrim, J.,** Mini-review: from in vitro to ex vivo studies: an overview of alternative methods for the study of medical biofilms, Biofouling, 2020, 36, 9, 1129-1148.
14. **Cerca, F., Andrade, F., França, Â., Andrade, E.B., Ribeiro, A., Almeida, A.A., Cerca, N., Pier, G., Azeredo, J., Vilanova, M.,** *Staphylococcus epidermidis* biofilms with higher proportions of dormant bacteria induce a lower activation of murine macrophages, Journal of Medical Microbiology, 2011, 60, 12, 1717-1724.
15. **Doll, K., Jongsthaphongpun, K.L., Stumpp, N.S., Winkel, A., Stiesch, M.,** Quantifying implant-associated biofilms: Comparison of microscopic, microbiologic and biochemical methods, Journal of Microbiological Methods, 2016, 130, 61-68.
16. **Ercolini, D., Russo, F., Blaiotta, G., Pepe, O., Mauriello, G., Villani, F.,** Simultaneous detection of *Pseudomonas fragi*, *P. lundensis*, and *P. putida* from meat by use of a multiplex PCR assay targeting the carA gene, Applied and Environmental Microbiology, 2007, 73, 7, 2354-2359.

17. **Ferrocino, I., Ercolini, D., Villani, F., Moorhead, S.M., Griffiths, M.W.,** *Pseudomonas fragi* strains isolated from meat do not produce N-acyl homoserine lactones as signal molecules, Journal of Food Protection, 2009, 72, 12, 2597-2601.
18. **Flemming, H.C., Wingender, J.,** The biofilm matrix, Nature reviews, Microbiology, 2010, 8, 9, 623-633.
19. **Grand, I., Bellon-Fontaine, M.N., Herry, J.M., Hilaire, D., Moriconi, F.X., Naïtali, M.,** Possible overestimation of surface disinfection efficiency by assessment methods based on liquid sampling procedures as demonstrated by in situ quantification of spore viability, Applied and Environmental Microbiology, 2011, 77, 17, 6208-6214.
20. **Hall-Stoodley, L., Stoodley, P., Kathju, S., Høiby, N., Moser, C., Costerton, J.W., Moter, A., Bjarnsholt, T.,** Towards diagnostic guidelines for biofilm-associated infections, FEMS Immunology and Medical Microbiology, 2012, 65, 2, 127-145.
21. **Huang, Y., Chakraborty, S., Liang, H.,** Methods to Probe the Formation of Biofilms: Applications in Foods and Related Surfaces, Analytical Methods, 2020, 12, 416-432.
22. **Kırmusaoğlu, S.,** Antimicrobials, Antibiotic Resistance, Antibiofilm Strategies and Activity Methods, IntechOpen, London, 2019.
23. **Lebeaux, D., Chauhan, A., Rendueles, O., Beloin, C.,** From in vitro to in vivo Models of Bacterial Biofilm-Related Infections, Pathogens (Basel, Switzerland), 2013, 2, 2, 288-356.
24. **Li, L., Mendis, N., Trigui, H., Oliver, J.D., Faucher, S.P.,** The importance of the viable but non-culturable state in human bacterial pathogens, Frontiers in Microbiology, 2014, 5, 258.
25. **McDougald, D., Rice, S.A., Barraud, N., Steinberg, P.D., Kjelleberg, S.,** Should we stay or should we go: mechanisms and ecological consequences for biofilm dispersal, Nature reviews, Microbiology, 2011, 10, 1, 39-50.
26. **Tîrziu, E., Moza, A.C.,** Exploring the current knowledge concerning the bacterial biofilm formation and adhesion to surfaces, Scientifical Papers: Veterinary Medicine Timisoara, 2021, 54, 3, 164-173.
27. **Nocker, A., Mazza, A., Masson, L., Camper, A.K., Brousseau, R.,** Selective detection of live bacteria combining propidium monoazide sample treatment with microarray technology, Journal of Microbiological Methods, 2009, 76, 3, 253–261.
28. **Nwaneshiudu, A., Kuschal, C., Sakamoto, F.H., Anderson, R.R., Schwarzenberger, K., Young, R.C.,** Introduction to confocal microscopy, The Journal of Investigative Dermatology, 2012, 132, 12.
29. **Ommen, P., Zobek, N., Meyer, R.L.,** Quantification of biofilm biomass by staining: Non-toxic safranin can replace the popular crystal violet, Journal of Microbiological Methods, 2017, 141, 87-89.

30. **Pantanella, F., Valenti, P., Natalizi, T., Passeri, D., Berlutti, F.,** Analytical techniques to study microbial biofilm on abiotic surfaces: pros and cons of the main techniques currently in use, Annali di igiene: medicina preventiva e di comunita, 2013, 25, 1, 31-42.
31. **Peeters, E., Nelis, H.J., Coenye, T.,** Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates, Journal of Microbiological Methods, 2008, 72, 2, 157-165.
32. **Romanova, N.A., Gawande, P.V., Brovko, L.Y., Griffiths, M.W.,** Rapid methods to assess sanitizing efficacy of benzalkonium chloride to *Listeria monocytogenes* biofilms, Journal of Microbiological Methods, 2007, 71, 3, 231-237.
33. **Roy, R., Tiwari, M., Donelli, G., Donelli, V.,** Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action, Virulence, 2018, 9, 1, 522-554.
34. **Schlafer, S., Meyer, R.L.,** Confocal microscopy imaging of the biofilm matrix, Journal of Microbiological Methods, 2017, 138, 50-59.
35. **Sheridan, G.E., Masters, C.I., Shallcross, J.A., MacKey, B.M.,** Detection of mRNA by reverse transcription-PCR as an indicator of viability in *Escherichia coli* cells, Applied and Environmental Microbiology, 1998, 64, 4, 1313-1318.
36. **Shunmugaperumal, T.,** Biofilm Eradication and Prevention: A Pharmaceutical Approach to Medical Device Infections, John Wiley & Sons Inc., New Jersey, 2010.
37. **Sidrim, J.J., Amando, B.R., Gomes, F.I.F., do Amaral, S.M.S.G., de Sousa, P.C.P., Ocadaque, C.J., Brilhante, R.S.N., Cordeiro, R.A., Rocha, M.F.G., Castelo-Branco, D.S.C.M.,** Chlorpromazine-impregnated catheters as a potential strategy to control biofilm-associated urinary tract infections, Future Microbiology, 2019,14, 12, 1023-1034.
38. **Singh, S., Singh, S.K., Chowdhury, I., Singh, R.,** Understanding the Mechanism of Bacterial Biofilms Resistance to Antimicrobial Agents, Open Microbiology Journal, 2017, 11, 53-62.
39. **Tang, H.J., Chen, C.C., Ko, W.C., Yu, W.L., Chiang, S.R., Chuang, Y.C.,** In vitro efficacy of antimicrobial agents against high-inoculum or biofilm-embedded meticillin-resistant *Staphylococcus aureus* with vancomycin minimal inhibitory concentrations equal to 2 µg/mL (VA2-MRSA), International Journal of Antimicrobial Agents, 2011, 38, 1, 46-51.
40. **Warwick, S., Wilks, M., Hennessy, E., Powell-Tuck, J., Small, M., Sharp, J., Millar, M.R.,** Use of quantitative 16S ribosomal DNA detection for diagnosis of central vascular catheter-associated bacterial infection, Journal of Clinical Microbiology, 2004, 42, 4, 1402-1408.
41. **Whitehead, K.A., Benson, P., Verran, J.,** Differential fluorescent staining of *Listeria monocytogenes* and a whey food soil for quantitative analysis of surface hygiene, International Journal of Food Microbiology, 2009, 135, 1, 75-80.

42. **Wickramasinghe, N., Ravensdale, J., Coorey, R., Gary, A., Chandry, P.,** Transcriptional profiling of biofilms formed on chilled beef by psychrotrophic meat spoilage bacterium, *Pseudomonas fragi* 1793, Biofilm, 2021, 3, 7.
43. **Wickramasinghe, N.N., Ravensdale, J.T., Coorey, R., Dykes, G.A., Scott Chandry, P.,** In situ characterisation of biofilms formed by psychrotrophic meat spoilage pseudomonads, Biofouling, 2019, 35, 8, 840-855.
44. **Wilson, C., Lukowicz, R., Merchant, S., Valquier-Flynn, H., Caballero, J., Sandoval, J., Okuom, M., Huber, C., Brooks, T.D., Wilson, E., Clement, B., Wentworth, C.D., Holmes, A.E.,** Quantitative and Qualitative Assessment Methods for Biofilm Growth: A Mini-review, Research & reviews, Journal of Engineering and Technology, 2017, 6, 4.
45. **Xie, Z., Thompson, A., Kashleva, H., Dongari-Bagtzoglou, A.,** A quantitative real-time RT-PCR assay for mature C. albicans biofilms, BMC Microbiology, 2011, 11, 93.
46. **Zambori, C., Cumpănășoiu, C., Huţu, I., Liker, M., Gurban, C., Tîrziu, E.,** The Pathogenic Microflora and Microbial Interactions in Oral Cavity in Dogs, Scientific Papers: Animal Science and Biotechnologies, 2014, 47, 1, 131-134.
47. **Zambori, C., Morvay, A.A, Gurban, C., Liker, M., Tanasie, G., Colibar, O., Cumpănășoiu, C., Șonea, C.G., Șonea, A.C., Tîrziu, E.,** Biofilm formation of Staphylococcus, Streptococcus, Pasteurella and Neisseria strains, Romanian Biotechnological Letters, 2015, 20, 4, 10718-10726.
48. **Zambori, C., Morvay, A.A., Sala, C., Licker, M., Gurban, C., Tanasie, G., Tirziu, E.,** Antimicrobial effect of probiotics on bacterial species from dental plaque, Journal of Infection in Developing Countries, 2016, 10, 3, 214-221.
49. **Zambori, C., Tîrziu, E., Nichita, I., Cumpănășoiu, C., Gros, R.V., Seres, M., Mladin, B., Mot, D.,** Biofilm Implication in Oral Diseases of Dogs and Cats, Scientific Papers: Animal Science and Biotechnologies, 2012, 45, 2.

HEMATOLOGICAL AND BIOCHEMICAL PROFILE IN GOATS FROM ALBA COUNTY DURING THE POST-PARTUM PERIOD

OLAR L.E., PURDOIU R.C., CODEA R.A., TOMOIAGĂ V.D., LĂCĂTUȘ C.M., MÂRZA S.M., LĂCĂTUȘ R.

University of Agricultural Sciences and Veterinary Medicine,
Faculty of Veterinary Medicine, 400000,
Calea Mănăștur No. 3-5, Cluj-Napoca, Romania
E-mail: robert.purdoiu@usamvcluj.ro

Summary

The aim of the present study was to investigate the impact of the pregnancy on hematobiochemical parameters in a number of 20 goats. Blood samples were collected during the postpartum period (21 days), (n=20 goats, including 12 Carpathian goats and 8 hybrids of Carpathian and French-Alpine goats). Non-pregnant adult goats (n=20) were used as controls. The hematobiochemical parameters: hemoglobin (Hb), blood serum glucose (GL), total protein (TP), albumin (AL), uree (UR), total cholesterol (TC), creatinine (CR), alanine aminotransferase (ALT), alkaline phosphatase (AP) and gamma-glutamyl transferase (GGT) were measured using commercial kits. Also the levels of minerals such as: calcium (Ca), phorphorus (P) and iron (Fe) were investigated. The result showed that the mean values for Hb, ALT and GGT were significantly higher in post-partum stages when compared with controls. In contrast, we observed a decrease in TP, AL, Ca, P and Fe^{3+} during the post-partum period. In conclusion, the haemato-biochemical profile of the goats during the post-partum period suffered some changes when compared with the non-pregnant goats. These variations in the metabolic profile are representative for monitoring the goats' nutritional status and health.

Keywords: biochemistry, goats, post-partum period, metabolic profile.

In both animal and human medical practice, the haemato-biochemical profile is used to predict some metabolic diseases, to assess the nutritional status and to monitories the health condition of animals (1, 16). Goats are one of the species with the unique ability to adapt and survive in varied environmental conditions, and implicitly to present some major variations in the haemato-biochemical profile in function of their physiological condition (6, 12). Thus, in literature, it was impossible to elaborate a universal metabolic profile test for all breeds of goats (2).

Special conditions such as pregnancy, parturition and lactation could influence and perturb the metabolism and therefore affect the hematological and biochemical parameters (2, 12, 17). The kidding period is one of the most demanding periods for animals in general and for the goats in particular, in the literature it is underlined that the fetal growth, lactogenesis and low dietary intake demand some high nutrient requirements (8, 19). Moreover, there was a special interest in using the haematobiochemical profile as a marker in the evaluation of nutrition efficiency in goats (4, 13). Apart from this, the haematobiochemical profile is also used to predict the apparition of some metabolic disorders that are more susceptible to install

during the peri-parturient period in goats as: peri-parturient hypocalcemia, pregnancy toxemia, and hypomagnesemia (3, 5).

Therefore, it is important to correct evaluate and understand the haemato-biochemical profile during and especially after parturition in order to prevent the apparition of some metabolic diseases. In this context, the objective of this study was to evaluate the hematological and biochemical parameters in the third week after parturition in goats raised in Alba County.

Materials and methods

Animals

The study was conducted during 2020 in a farm from Baia de Aries in Alba County. The haemato-biochemical profile was investigated in 20 non-pregnant goats and in 20 goats during the post-partum period (21 days). The goats were aged between 3 and 5 years and weighing 45-55 kg. Prior to blood samples collection, a clinical examination was practiced in both groups of goats. Therefore, firstly, we investigated their general behaviour and secondly, we performed the auscultation of the heart, lungs, rumen and intestine in order to find some abnormal sounds and to determine their heart and respiratory rate. We also determined their body temperature. The goats' daily diet was composed mainly of posture, hay and 0,2 kg/goat of concentrate. They also received fresh water and mineral blocks. In all goats, the feed and water withdrawal were carried out 12 hours prior to blood sampling.

Blood sampling and analysis

The blood samples were taken from the goats during the non-pregnancy and postpartum period. We collected 2 ml of blood in EDTA tubes for haematological analyses and 2 ml in plain tubes (serum) for the biochemical analyses. All blood samples were centrifuged at a maximum speed of 4000 rot/min for 3 min. The haematochemical analyses were carried out in triplicate, using commercially available kits according to the manufacturer's instructions. Therefore, the biochemical analyses were realized with UV-VIS Screen Master Toreh. Determination of hematological parameters (HGB, HCT, Ery, Prot and Alb and Y-glob) in whole blood of goats was carried out on an automatic three diff hematology analyzer Sysmex PocH-100iV.

Data analysis

The collected data regarding the concentration of hematological and biochemical parameters of goats' blood were presented as mean value and standard error of mean. The differences between groups were calculated with a Turkey test at the level of $p< 0,005$. All data were analyzed with the IBM SPSS statistics version 26.

Results and discussions

The results show that significant differences were observed in the blood concentration of Hg, HCT, Ery, TP and Al ($p \leq 0.05$) in the postpartum group of goats when compared with the non-pregnant group (Table 1). In contrast, no differences were found in the blood concentration of Y-glob for the evaluated groups of goats. Concerning the biochemical parameters evaluation, a significant increase was observed in the blood UR and GL concentration ($p \leq 0.05$) (Table 2). On the other hand, a significant decrease was evidenced as regards blood Cr, ALT, AST, GGT, Ca, P and Fe concentration ($p \leq 0.05$) (Table 2).

Table 1

Mean values (*x*) and standard deviations (± SD) of hematological parameters in goats at 21 days of the periparturient period

Parameter	Postpartum goats (n=20)	Non-pregnant goats (n=20)
Hb (g/dl)	10.36±0.70	9.18±0.94*
HCT (%)	31.6±2.06	29.30±2.38**
Ery (mil/mm^3)	5.03±0.51	10.55±1.97**
TP (g/dl)	5.42±0.84	6.54±0.63**
Al (g/dl)	2.37±0.43	2.95±0.30*
Y-glob (g/dl)	2.21±0.63	2.07±0.26

Statistically significant differences: * ($P \leq 0.05$) and ** ($P \leq 0.01$)

These results are in accordance with the recent literature. Therefore, some research has shown lower levels of blood calcium, phosphorus and total proteins in the first weeks after parturition (17, 19). The lowering levels of calcium and phosphorus in the first weeks after parturition are explained by a decline in the numbers of receptors for 1,25-dihydroxyvitamin D in the goats' intestine (9). Moreover, this drop in blood calcium levels could also be associated with the increased milk production and colostrum synthesis during the first weeks after parturition (17, 19). Other changes were observed in the hematological profile and included the blood Hb, HCT and Al. Our results showed an increase in the levels of Hb and HCT and a decrease in the Al concentration. However, these changes may serve as an indicator of the stress associated with the parturition and the relactation (7, 17). Another variation in the biochemical profile was observed in the blood glucose levels of goats. According to some recent studies (19, 20), our results might not interfere with the animal's physiology as the normal blood glucose level in goats range within 50 and 74 mg/dL. However, in the present study, a significant increase in blood glucose level was recorded in the post-partum goats when compared to the control group. This may be due to the increased cortisol levels, as cortisol stimulates hepatic gluconeogenesis and further increases circulating glucose (10).

With respect to significantly lowers levels of proteins in goats during the post-partum period, in literature this phenomenon was associated with the immunoglobulin production to colostrum, fetus growing and also due to a possible protein deficiency in the goats' diet (11, 19).

Some physiological variations occur also in the serum blood levels of enzymes AST and GGT. Therefore, we observed an increase in the serum activity of GGT and a decrease of AST activity in post-partum group of goats. In some recent studies, the serum activity of AST and GGT was evaluated in goats and cows during the transition period (three weeks before to three weeks after the parturition) (17, 18). Comparable to our results, the results showed an increase in both AST and GGT activity, suggesting a possible accumulation of triglycerides in the liver. However, in order to explain and confirm these results the examination of triglycerides' accumulation within the liver is mandatory.

The serum cholesterol level also suffered some changes in goats at three weeks after parturition. Hence, the cholesterol levels were higher than in controls, but without a statistical significance. These variations were observed also in other studies and were associated with an increase in the lipoprotein and hepatic lipase activity (14, 15).

Table 2

Mean values (*x*) and standard deviations (±SD) of biochemical parameters in goats at 21 days of the periparturient period

Parameter	Postpartum goats (n=20)	Non-pregnant goats (n=20)
UR (mg/dl**)**	46.5±12.57	38.10±3.44**
CR (mg/dl**)**	0.739±0.14	0.95±0.122**
GL (mg/dl**)**	63.92±10.09	49.23±4.23**
TC (mg/dl**)**	113.34±28.51	109.29±11.16
ALT (U/L**)**	16.55±9.67	12.21±2.09*
AST (U/L**)**	30.72±5.49	65.42±5.07**
PAL (U/L**)**	178.11±126.32	127.76±17.07
GGT (U/L**)**	85.68±19.51	35.23±5.55**
Ca (mg/dl)	7.96±1.62	10.27±1.36*
P (mg/dl)	5.42±1.81	6.93±1.41*
Mg (mg/dl)	2.07±0.34	2.08±0.53
Fe^{+3} (mmg)	87.24±30.72	101.98±10.67*
CK (U/L)	68.92±26.76	71.80±8.53

Statistically significant differences: * ($P \leq 0.05$) and ** ($P \leq 0.01$)

Conclusions

In conclusion, the haematobiochemical profile of the examined goats during the post-partum period suffered some significant abnormalities. Therefore, the hematological changes concern the level of HCT, Hb, AI, while the abnormalities in the biochemical parameters refer to changes in blood UR, GL Cr, ALT, AST, GGT, Ca, P and Fe concentration. These changes can be used to evaluate their health status and to predict the apparition of some metabolic disorders and obesity.

Bibliography

1. **Antunović, Z., Marić, I., Klir, Ž., Šerić, V., Mioč, B., Novoselec, J.,** Haemato-biochemical profile and acid-base status of Croatian spotted goats of different ages, Archives Animal Breeding, 2019, 24, 62, 455-463.
2. **Azab, M.E, Abdel-Maksoud, H.A.,** Changes in some hematological and biochemical parameters during prepartum and postpartum periods in female Baladi goats, Small Ruminant Research, 1999, 34, 77-85.
3. **Bayoumi, Y.H., Behairy, A., Abdallah, A.A., Attia, N.E.,** Peri-parturient hypocalcemia in goats: Clinical, hematobiochemical profiles and ultrasonographic measurements of postpartum uterine involution, Veterinary World, 2021, 14, 558-568.
4. **Belewu, M.A., Ogunsola, F.O.,** Haematological and serum indices of goat fed fungi treated *Jatropha curcas* kernel cake in a mixed ration, Journal of Agricultural Biotechnology and Sustainable Development, 2010, 2, 35-38.
5. **Brozos, C., Mavrogianni, V.S., Fthenakis, G.C.,** Treatment and control of peri-parturient metabolic diseases:pregnancy toxemia, hypocalcemia, hypomagnesemia, Veterinary Clinics of North America: Food Animal Practice, 2011, 27, 105-113.
6. **Cepeda-Palacios, R., Fuente-Gómez, M.G., Ramírez-Orduña, J.M., García-Álvarez, A., Llinas-Cervantes, X., Angulo, C.,** Effects of pregnancy and post-kidding stages on haematochemical parameters in cross-bred goats, Journal of Applied Animal Research, 2018, 46, 269-273.
7. **El-Ghoul, W., Hofmann, W., Khamis, Y., Hassanein, A.,** Relationship between claw disorders and the peripartal period in dairy cows, Prakt Tierarzt, 2000, 81, 862-868.
8. **Gandra, J.R., Mingoti, R.D., Barletta, R.V., Takiya, C.S., Verdurico, L.C., Freitas, J.E.,** Effects of flaxseed, raw soybeans and calcium salts of fatty acids on apparent total tract digestibility, energy balance and milk fatty acid profile of transition cows, Animal: An International Journal of Animal Bioscience, 2016, 10, 1303-1310.
9. **Goff, J.P.,** Pathophysiology of Calcium and Phosphorus Disorders, Veterinary Clinics of North America: Food Animal Practice, 2000, 16, 319-337.

10. **Ingvartsen, K.L, Andersen, J.B.,** Integration of metabolism and intake regulation:a review focusing on periparturient animals, Journal of Dairy Science, 2000, 83, 1573-1597.
11. **Jainudee, M. R., Hafez, E.S.E.,** Gestation, prenatal physiology and parturition, Philadelphia: Lea and Febige, 1994.
12. **Manat, T.D., Chaudhary, S.S., Singh, V.K., Patel, S.B., Puri, G.,** Hematobiochemical profile in Surti goats during post-partum period, Veterinary World, 2016, 9, 19-24.
13. **Mohammed, S.A., Mohammed A.,R., Omar A.E., Albert S., Al-Gallaf W.M.,** Biochemical and hematological profile of different breeds of goat maintained under intensive production system, African Journal of Biotechnology, 2016, 15, 1253-1257.
14. **Nazifi, S., Saeb, M., Ghavami, S.M.,** Serum lipid profile in Iranian fat-tailed sheep in late pregnancy, at parturition and during the post-parturition period, Journal of Veterinary Medicine. A, Physiology, Pathology, Clinical Medicine, 2002, 49, 9-12.
15. **Smith, J.L., Lear, S.R., Forte, T.M., Ko, W., Massimi. M., Erickson, S.K.,** Effect of pregnancy and lactation on lipoprotein and cholesterol metabolism in the rat, Journal of Lipid Research,1998, 39, 2237-49.
16. **Soul, W., Mupangwa, J., Muchenje, V., Mpendulo, T.C.,** Biochemical indices and heamtological parameters of goats fed *lablab purpureus* and *vigna unguiculata* as supplements to a *chloris gayana* basal diet, Veterinary and Animal Science, 2019, 8, 100073.
17. **Tharwat, M., Ali, A., Al-Sobayi, F.,** Hematological and biochemical profiles in goats during the transition period, Comparative Clinical Pathology, 2015, 24, 1-7.
18. **Tharwat, M., Oikawa, S., Buczinski, S.,** Ultrasonographic prediction of hepatic fat content in dairy cows during the transition period, Journal of Veterinary Science & Technology, 2012, 3, 1.
19. **Tosto, M.S.L., Santos, S.A., Filho, R.D.C.P., Rodrigues, T.C.G.C., Nicory, I.M.C., de Carvalho, G.G.P., Bittencourt, R.F., Ayres, M.C.C., Pereira, T.C.J.,** Metabolic and behavior changings during the transition period as predictors of calving proximity and welfare of dairy goats, Veterinary Animal Science, 2021, 11, 100168.
20. **Kaneko, J.J., Harvey, J.W., Bruss, M.L.,** Clinical biochemistry of domestic animals, New York: Academic Press, 2008.

EVALUATION OF THE PARASITIC LOAD IN FALLOW DEER (*DAMA DAMA L.*) FROM ARAD COUNTY

POPOVICI D.C.[1], MEDERLE N.[2], MARIN A.M.[2], MORARU M.M.F.[2], ALBU KAYA M.G.[3], IONESCU O.[1]

[1]Transilvania University Brasov, Forestry Faculty
[2]Banat's University of Agricultural Sciences and Veterinary Medicine" King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645, No.119, Calea Aradului, Timisoara, Romania
[3]Natl Res & Dev Inst Text & Leather, Div Leather & Footwear Res Inst, Dept Collagen, Bucharest 031215, Romania
E-mail: danpopovici30@yahoo.com

Summary

The fallow deer (*Dama dama L.*) is a notable representative of the *Cervidae* family in the Romanian fauna, particularly of the genus *Dama,* being an allogeneic species reintroduced into the country's fauna during antiquity by the Romans. A species with a high gregarious instinct, the fallow deer is directly dependent on the conditions offered by the habitat, and from this interaction, between the need of the species and the trophic offer, results in relations of interference and interspecific conditionality, respectively competition between individuals of the same species or between individuals of different species. One of the types of interspecific relationship encountered in the fallow deer species is predation. Exhaustively simplistic predation can be defined as the phenomenon by which some living individuals eat/consume all or part of other living individuals, excluding here detritivores and necrophages. One form of predation is parasitism. In this context, the purpose of the present study was to identify the presence of endoparasites in fallow deer from Arad County using the coprologic methods. We identified, morulated strongilid eggs (gastrointestinal nematodes), *Nematodirus spp.* eggs, oncosphers of cestodes, eggs of *Paramphistomum spp.* and eggs of *Gongylonema spp*. In conclusion, the increased prevalence of endoparasitism with the risk of infestation of the domestic ruminants, in fallow deer, indicates the existence of an epidemiological context favorable to the development of parasitic elements in Arad County and a warning for domestic animal breeders.

Keywords: fallow deer, endoparasitism, Arad County.

The fallow deer (*Dama dama L.*) is a notable representative of the *Cervidae* family in the Romanian fauna, particularly of the genus *Dama,* being an allogeneic species reintroduced into the country's fauna during antiquity by the Romans (23).

A species with a high gregarious instinct, the fallow deer is directly dependent on the conditions offered by the habitat, and from this interaction, between the need of the species and the trophic offer, results in relations of

interference and interspecific conditionality, respectively competition between individuals of the same species or between individuals of different species. One of the types of interspecific relationship encountered in the fallow deer species is predation. Exhaustively simplistic predation can be defined as the phenomenon by which some living individuals eat/consume all or part of other living individuals, excluding here detritivores and necrophages. One form of predation is parasitism (22).

Gastrointestinal helminth parasites infecting ungulates have profound impacts on their productivity. Parasites infecting such ungulates include coccidian parasites, nematodes, cestodes and trematodes (2, 3, 6, 18).

The wild animals serve as a reservoir of various intestinal parasites including nematodes and trematodes and interactions of these wild animals in different ecosystems execute considerable role in hauling of parasites among different species (4, 5, 9, 16).

Materials and methods

The study was conducted a 30 samples of fallow deer droppings from 8 different hunting grounds from Arad County. Animals are maintained on dry pastures, but also wet, with / without swampy land (Fig. 1).

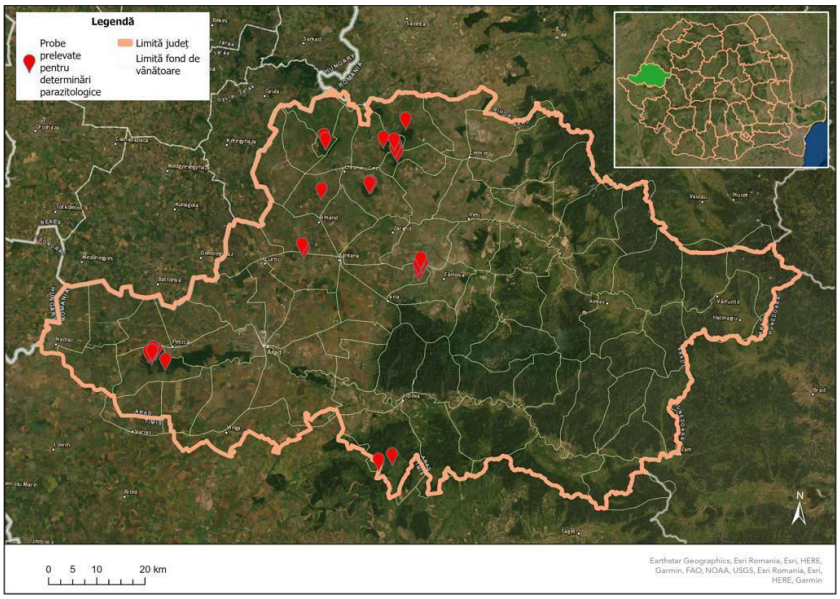


Fig. 1. Map of Arad County

Between 50 and 150 g of freshly discarded faces were collected or directly from the rectum of the goats under study. The samples were collected in tubs and refrigerated until processing (Fig. 2, Fig. 3).

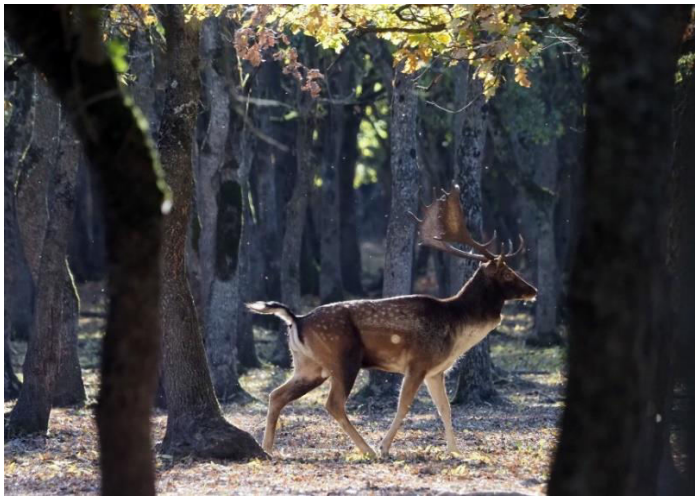


Fig. 2. Fallow deer (*Dama dama L.*)

Fig. 3. Fallow deer droppings

The samples were transported and examined in the Parasitology and Parasitic Diseases Clinics of the Faculty of Veterinary Medicine, Timisoara and were processed by the following methods (7, 8):

- Qualitative method - identification of the parasitic load of the whole flock with light eggs of nematodes, cestodes, protozoan oocysts;
- Polyvalent method (of successive washes) - identification of the presence of trematode eggs;
- Baermann method - highlighting the parasitism with pulmonary nematodes larvae (Fig. 2).

Results and discussions

The results of the coprological examination performed by the flotation method revealed the presence of the following parasitic elements: morulated eggs (gastrointestinal nematodes, *Nematodirus spp.*), oviger proglots (cestodes oncosfers) and eggs of *Gongylonema spp*. (Fig. 4 - Fig. 7).

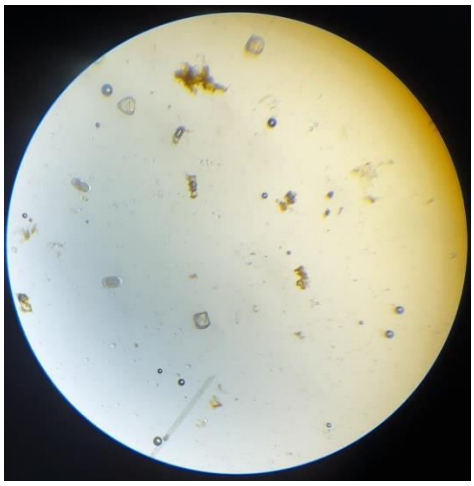


Fig. 4. Nematodes eggs

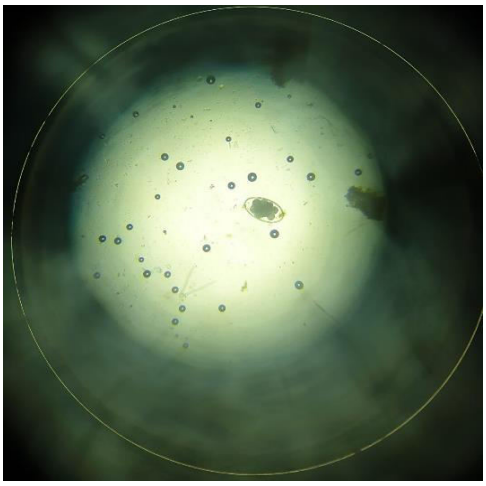


Fig. 5. *Nematodirus spp*. egg

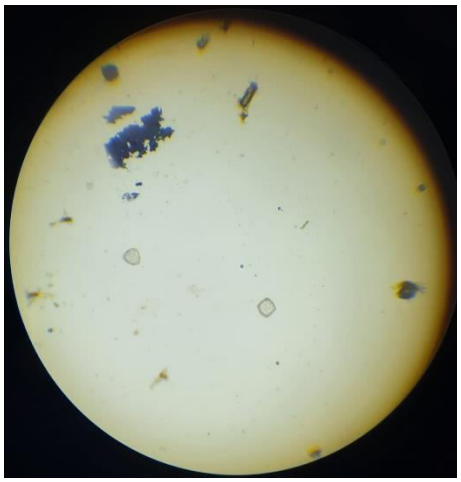


Fig. 6. Oviger proglots (cestodes oncosfers)

Fig. 7. *Paramphistomum spp. e*gg

The results of the coprological examination performed by the sedimentation method, respectively, revealed the absence of parasitic elements belonging to the genera *Fasciola*, *Dicrocelium spp*. The only parasitic elements identified following this coprological method were the heavy eggs of *Paramphistomum spp.* (Fig. 8).

The results of the coprological examination performed by the Baermann method revealed the absence of parasitic elements belonging to the genera *Dictiocaulus spp*.

The coprological examinations performed on several 30 samples of revealed the following results:

- Presence of gastrointestinal nematodes eggs - 20/30 samples collected (66.7 %);
- Presence of *Gongylonema spp*. eggs - 5/30 samples collected (17%);
- Presence of cestode oncospheres - 1/30 samples collected (3.3%);
- The presence of *Paramphistomum spp*. eggs - 3/30 samples collected (10%).

Aspects related to the distribution and spread of internal parasites in domestic and wild ruminants and carnivores, specificity of areas, presence or absence of certain factors that determine different manifestations of endo-parasitosis, host-parasite relationship, links between biotic and abiotic components involved in biological cycles have been aimed in research performed in our country and abroad (10, 11, 12, 13, 14, 19).

Survival strategies of parasites and the impact of endo-parasitism on fallow deer life are subjects of several studies (1, 15, 17, 20).

A study conducted in south of Northrhine-Westfalia, Germany on a herd of

64 fallow deer reveals a prevalence of 19 species of gastrointestinal nematodes and one lungworm species were. The prevalence of nematodes in the abomasum, small intestine and large intestine was 93.8 %, 57.8 % and 87.5 %, respectively. The most prevalent species were *Spiculopteragia asymmetrica* (84.4%), *Oesophagostomum sikae* (71.9 %), *Spiculopteragia bohmi* (45.3 %), *Nematodirus roscidus* (37.5 %), *Capillaria bovis* and *Oesophagostomum venulosum* (35.9 % both), *Ostertagia leptospicularis* (34.4 %) and *Apteragia quadrispiculata* (25.0 %). *Trichostrongylus askivali*, *Trichostrongylus capreoli*, *Nematodirus battus*, *Nematodirus roscidus* and *Ascaris suum* (fourth and fifth stage larvae) were recorded for the first time as parasites of German fallow deer (21).

Conclusions

In a fallow deer dropping collected from Arad County hunting grounds and examined by coprological methods we identified presence of gastrointestinal nematodes eggs (66.7 %), *Gongylonema spp*. eggs (17%), cestode oncospheres (3.3%) and the presence of *Paramphistomum spp*. eggs (10%).

The identification of digestive endoparasites in a considerable percentage reveals their impact on the health of the fallow deer, but also warns about the possible risk of infestation of the environment and implicitly that of other possible host animals.

Acknowledgments

This scientific paper was carried out in the Laboratory of Parasitology and Parasitic Disease at the Faculty of Veterinary Medicine Timisoara, Laboratory within the Animal Hygiene and Pathology Research Center/ BUASVM Timisoara.

References

1. **Borkovcova, M., Langrova, I., Totkova, A.,** Endoparasitoses of fallow deer (*Dama dama*) in game-park in South Moravia (Czech Republic), Helminthologia, 2013, 50, 1, 15-19.
2. **Constantin, N., Darabus, Gh.,** Tratat de Medicină Veterinară, Ed. Risoprint, Cluj Napoca, 2014.
3. **Dar, J.A., Fazili, M.F., Bhat, B.A., Ahmad, R., Wani, I.N.,** Prevalence of Gastrointestinal Helminth Parasites of Himalayan goral (Naemorhedus goral) in Kazinag National Park, Jammu and Kashmir, India, Dian Journal of Animal Research, 2022, 56, 5, 587-593.
4. **Dărăbuş, Gh.,** Controlul parazitologic în helmintozele ierbivorelor - partea I, Revista Română Medicină Veterinară, 2003, 13, 2, 70-81.
5. **Dărăbuş, Gh.,** Controlul parazitologic în helmintozele ierbivorelor – partea a II-a, Revista Română Medicină Veterinară, 2004, 14, 1, 58-66.

6. **Dărăbuş, Gh., Oprescu, I., Morariu, S. Mederle, N.,** Ediție revizuită, Parazitologie și Boli Parazitare, Ed. Mirton, Timişoara, 2019.
7. **Dărăbuş, Gh., Oprescu, I., Morariu, S. Mederle, N., Ilie, M.,** Ghid practic în bolile parazitare, vol. I, Ed. Agroprint, Timişoara, 2013.
8. **Dărăbuş, Gh., Oprescu, I., Morariu, S. Mederle, N., Ilie, M.,** Ghid practic în bolile parazitare, vol. II, Ed. Agroprint, Timişoara, 2013.
9. **Dărăbuş, Gh.,** Principii generale de control al helmintozelor la ovine, Revista Română Medicină Veterinară, 1998, 4, 17-29.
10. **Hora, F.Ș., Mederle, N., Badea, C., Tilibașa, E.M., Gartner, A., Ilie, M.S., Morariu, S., Dărăbuș, Gh.,** Prevalence of gastrointestinal parasitoses in wild boars (*Sus scrofa*) in Arad County, Lucrari Stiintifice Medicina Veterinara Timisoara, 2015, 48, 3, 59-64.
11. **Hora, F.Ș., Mederle, N., Badea, C., Tilibașa, E.M., Ilie, M.S., Dărăbuș, Gh.,** Digestive parasite fauna in hare (*Lepus europaeus*) in Western Romania, Scientific Works Series C Veterinary Medicine, 2015, 61, 1, 138-141.
12. **Hora, F.Ș., Mederle, N., Ilie, M.S., Imre, M., Badea, C., Darabuș, Gh.,** The prevalence of gastrointestinal parasites in red foxes (*Vulpes vulpes*) in Bihor County, Lucrari Stiintifice Medicina Veterinara Timisoara, 2016, 49, 3, 68-73.
13. **Hora, F.Ş., Mederle, N., Popa, C., Badea, C., Imre, M., Ilie M.S., Dărăbuş, Gh.,** Endoparaziţi cu caracter zoonotic la vulpi (*Vulpes Vulpes*) în Judeţul Caraş-Severin, România, Revista Română Medicină Veterinară, 2016, 26, 4, 41-43.
14. **Hora, Ș., Genchi, C., Ferrari, N., Morariu, S., Mederle, N., Dărăbuș Gh.,** Frequency of gastrointestinal and pulmonary helminth infections in wild deer from western Romania Veterinary Parasitology: Regional Studies and Reports Volume, 2017, 8, 75-77.
15. **Iglodyova, A., Lazar, P., Curlik, J., Karolova, R., Ciberej, J., Bockova, E., Strkolcova, G.,** Observations on autochtonous liver flukes in wild ruminants in Slovakia, Helminthologia, 2017, 54, 4, 307-313.
16. **Komaromy, A.M.,** Day blind sheep and the importance of large animal disease models, Veterinary Journal, 2010, 185, 3, 241-242.
17. **Magdalek, J., Bourgoin, G., Vadlejch, J.,** Non-native Nematode Ashworthius sidemi Currently Dominates the Abomasal Parasite Community of Cervid Hosts in the Czech Republic, Frontiers in Veterinary Science, 2022, 9, 862092.
18. **Mitrea, I.L.,** Bolile parazitare la animale, Ed. Ceres, Bucureşti, 2002.
19. **Oprescu, I., Dărăbuș, G.H., Morariu, S., Mederle, N., Ilie, M.S., Hora, F., Rujici, D.,** Prevalence of helminthosis in hunting species Capreolus capreolus (Roe deer) and *Sus scrofa L.* (wild boar) in Câmpia Village sylvatic ecosystem, Caras-Severin County, Lucrari Stiintifice Medicina Veterinara Timisoara, 2015, 48, 3, 124-133.
20. **Rehbein, S., Lutz, W., Visser, M., Winter, R.,** Investigation of the parasite fauna of game animals of Northrhine-Westfalia, Endoparasites of fallow deer, Zeitschrift Fur Jagdwissenschaft, 2001, 47, 1, 1-16.

21. **Rehbein, S., Visser, M., Jekel, I., Silaghi, C.,** Endoparasites of the fallow deer (*Dama dama*) of the Antheringer Au in Salzburg, Austria, Wiener Klinische Wochenschrift, 2014, 126, 1, S37-S41.
22. **Sinclair R.E., Fryxell, J.,** Wildlife ecology, conservation and management, Garsington Road, Oxford, UK, 2006.
23. **Ueckermann, E., Hansen, P.,** Das Damwil, Ed. Verlag Paul Parey, Hamburg, 1971.

MORPHOLOGICAL FEATURES OF THE THORACIC LIMB BONES IN RED-NECKED WALLABY (*MACROPUS RUFOGRISEUS* – DESMAREST, 1817) - CASE STUDY

ROŞU P.M., GEORGESCU B., BELU C., DUMITRESCU I., MIHAI S.A., GUREŞOAE E.

University of Agronomic Sciences and Veterinary Medicine in Bucharest,
Faculty of Veterinary Medicine, 050097,
Splaiul Independenţei, No. 105, sector 5, Bucharest
E-mail: rosupetronelamihaela@yahoo.com

Summary

The study aims to describe the morphological features of the thoracic limb bones in red-necked wallaby (*Macropus rufogriseus*). The morphological characteristics found in these bones are useful for the diagnosis of the species. In order to perform this study, the bones of the thoracic limbs of a red-necked wallaby were used. The study of the thoracic limb bones, especially the long bones, led to the following conclusions: the scapular spine ends with an evident acromion, flanked by a reduced processus suprahamatus, the clavicle is present, flattened from side to side and extremities are widened, the humerus presents an supracondylar foramen, the lateral epicondylar crest is evident, in the proximal third of the radius body a crest for muscle insertion is observed, the olecranon tuberosity is evident and convex from side to side, the anconeal process is directed to the medial part, the first metacarpal bone is small in size.

Keywords: scapula, humerus, lateral epicondyle, olecranon tuberosity, radius.

The red-necked wallaby (*Macropus rufogriseus*) (Desmarest, 1817) is part of the Order *Marsupialia*, belonging to the *Macropodidae* Family, Genus *Macropus*. Kangaroos and wallabies are members of the *Macropodidae* Family. The family includes 16 genera, with more than 50 species, distributed in Australia, Tasmania, Papua New Guinea, the Bismarck Archipelago and New Zealand (where they were introduced by man) (2, 4, 5). On the Australian mainland, kangaroos are native, some of these species have disappeared completely, while the number of individuals of other species have been reduced, but the number of specimens is constantly increasing, with restrictive measures against the hunting of these species (1). In Europe, the red-necked wallaby (*Macropus rufogriseus*) is found especially in zoos, very rarely as a pet.

Regarding this species, the literature includes works on the morphology and morphometry of various components of the skeleton (thoracic limb, pelvic limb, skull, vertebral column), description of the limb and tail muscles and the way of moving for different species belonging to the family *Macropodidae* (3, 6, 9, 10, 12, 13, 14, 15). However, a detailed study identified some interesting anatomical aspects, some of them were found in felines (supracondylar foramen) or leporidae (the groove placed

on the medial face corresponding to the detachment of the scapular spine from the lateral face) (1, 7, 8, 11).

The study was performed on the bones of an appendicular skeleton of the thoracic limb from a specimen of red-necked wallaby (*Macropus rufogriseus*), and its purpose is to provide data that will help in conducting comparative studies between species belonging to the Order *Marsupialia* and among those belonging to the *Macropodidae* Family.

Materials and methods

The study material was represented by the bones of the thoracic limb that come from a specimen of red-necked wallaby (*Macropus rufogriseus*). The bones are part of the collection of the department of Anatomy.

The most interesting aspects were described and photographed. The description, identification and approval was made in accordance with the 2017 Nomina Anatomica Veterinaria (N.A.V.).

Results and discussions

The scapula has a relatively rectilinear scapular spine, that divides the lateral face into two fossae, which are called supraspinous and infraspinous fossae in a ratio of 1:2. The scapular spine terminates in an evident acromion, flanked by a small processus suprahamatus. An elongated and rough tuberosity can be observed in the central part of the scapular spine (Fig. 1).

The dorsal border of the scapula is convex and has a suprascapular cartilage. The cranial angle is rounded and the caudal angle is thickened. The middle part of the cranial border has an accentuated convexity. The neck of the scapula is much reduced in size. The caudal border is thickened.

The articular angle presents an elongated glenoid cavity that has a relatively oval shape, with the main axis being oriented in an antero-posterior direction. The supraglenoid tuberosity is evident and is detached from the margin of the glenoid cavity. A much reduced coracoid process is detached from the supraglenoid tuberosity in a cranio-medial direction. A first-order vascular foramen can be seen above the neck of the scapula (Fig. 2).

On the medial face of the scapula, there is an evident elongated subscapular fossa, crossed by muscle insertion lines and is provided with a groove corresponding to the place of detachment of the scapular spine from the lateral face (Fig. 3). The scapular notch is deep and narrow.

Fig. 1. Scapula of the red-necked wallaby (*Macropus rufogriseus*) – lateral face
1.Caudal angle; 2. The dorsal border of the scapula; 3. Cranial angle;
4. Tuberosity of the scapular spine; 5. Acromion; 6. Glenoid cavity;
7. Supraspinous fossa; 8. Infraspinous fossa; 9. Processus suprahamatus

Fig. 2. Scapula of the red-necked wallaby (*Macropus rufogriseus*) – distal extremity
1. Acromion; 2. Processus suprahamatus; 3. First-order vascular foramen;
4.Glenoid cavity; 5. Supraglenoid tuberosity; 6. Coracoid process

Fig. 3. Scapula of the red-necked wallaby (*Macropus rufogriseus*) – medial face
1. Scapular notch; 2. Coracoid process; 3. Acromion; 4. Glenoid cavity;
5. Groove corresponding to the place of detachment of the scapular spine on the lateral face.

The clavicle is slightly elongated, curved and flattened. Both extremities are wide and rounded (Fig. 4).

Fig. 4. Clavicle of the red-necked wallaby (*Macropus rufogriseus*)
1. Distal extremity; 2. Proximal extremity

The humerus has a relatively spheroidal articular head at the proximal extremity, caudally oriented (Fig. 5 A). Lateral to the humeral head is the large

tubercle, divided by a small incision into a cranial and a caudal portion. The lesser tubercle is medially situated and has a nipple-like appearance. The two tubercles are separated by an evident intertubercular groove, reaching the proximal third of the humerus body. An evident muscular crest descends from the greater tubercle to the distal third of the humerus (Fig. 5 B). The deltoid ridge is distinguished very well (Fig. 5 D).

At the distal extremity, a triangular condyle is seen on the lateral part. The trochlea has unequal margins. The medial margin is more developed. On the cranial face there is a reduced radial fossa which is arranged medially and a more evident coronoid fossa disposed laterally (Fig. 5 C). The crest of the lateral epicondyle is very high and slightly bent in the cranial direction. Medially, at the distal extremity of the humerus, an supracondylar foramen is observed, and distal to it, a tubercle with a rounded surface can be seen. The olecranon fossa is wide and shallow.

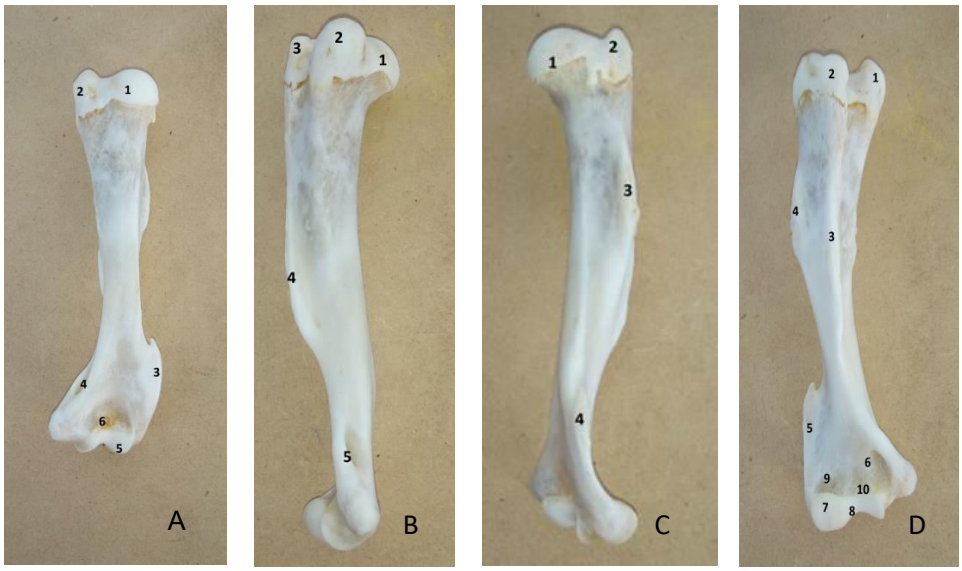


Fig. 5. Red-necked wallaby humerus (*Macropus rufogriseus*)
A. Caudal face – 1. Humeral head; 2. Lesser tubercle; 3. Lateral epicondyle ridge; 4. Supracondylar foramen; 5. Humeral trochlea; 6. Olecranon foramen.
B. Medial face – 1. Humeral head; 2. Lesser tubercle; 3. Greater tubercle; 4. Muscular crest; 5. Supracondylar foramen.
C. Cranial face – 1. Lesser tubercle; 2. Greater tubercle; 3. Muscular crest; 4. Deltoid ridge; 5.Lateral epicondyle ridge; 6. Supracondylar foramen; 7. Condyle; 8.Trochlea; 9. Coronoid fossa ;10. Radial fossa..
D. Lateral face - 1. Humeral head; 2. The greater tubercle; 3. The deltoid ridge; 4. Lateral epicondyle crest.

The radius, at the proximal extremity, presents a circular caudo-laterally glenoid cavity, and an evident tubercle of muscular insertion is observed (Fig. 6 A). A small muscular crest is observed in the proximal third of the radius body. At the distal extremity, on the cranial face, there are two evident sliding grooves for tendons. The medial styloid process is evident. The distal articular surface is represented by a wide and slightly elongated glenoid cavity.

Ulna has a slightly drawn cranial olecranon. Olecranon tuberosity is evident and convex from side to side. The anconeal process is directed to the medial part, the trochlear notch having the appearance of a semicircle (Fig. 6 B). Below the radial incision, there is an elongated fossa delimited by an evident ridge.

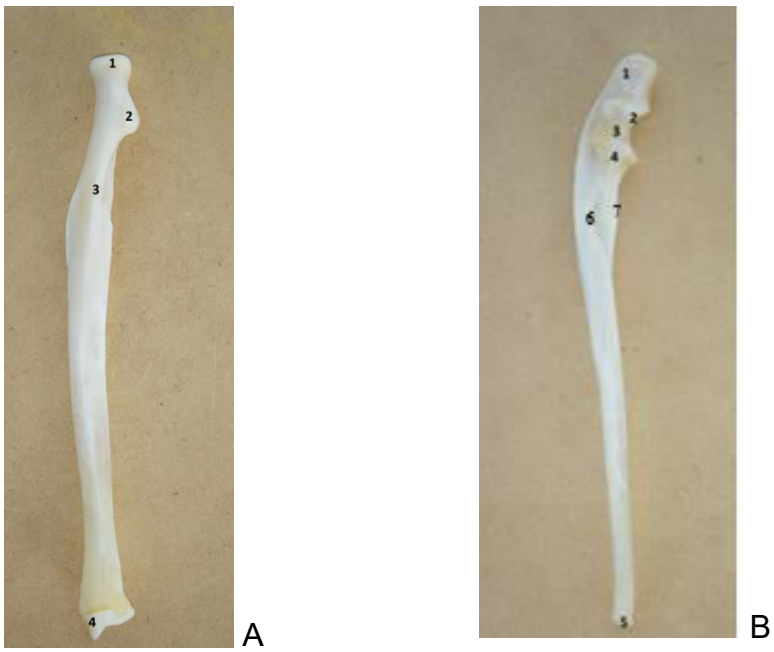


Fig. 6. **A.** Radius of the Red-necked Wallaby (*Macropus rufogriseus*) - 1. Articular cavity; 2. The tubercle for muscular insertion; 3. Muscular crest; 4. Radial styloid process.
B. Ulna of the Red-necked Wallaby (*Macropus rufogriseus*) - 1. Olecranon tuberosity; 2. The anconeal process; 3. Trochlear notch; 4. Radial notch; 5. The distal extremity of the ulna; 6. Fossa on the medial face; 7. Muscular crest

The carpal bones being short, are arranged in two rows: in first row can be found the pisiform, the triquetrum, and scapholunar, and in the second row the unciform, the capitate, the trapezium and the trapezoid.

The thoracic metapodium has five metacarpal bones. The smallest one being the first finger.

The first finger has two phalanges, and is shorter that the other four fingers that have three phalanges. The fingers end in non-retractable, extremely strong claws.

Conclusions

The scapular spine is evident, the acromion is very developed, but the processus suprahamatus is reduced. A first-order vascular foramen is present in the caudal part of the scapula's neck.

The clavicle is also present with flattened extremities.

The great humeral tubercle is divided into two parts by a small incision. An evident muscular crest descends from the greater tubercle to the distal third of the humeral body.

The crest of the lateral epicondyle is very high and slightly bent in the cranial direction. An supracondylar foramen is observed in the medial part of the distal extremity of the humerus, and below it there is a tubercle with a rounded surface.
In the proximal third of the radius body there is a small ridge of muscular insertion.

The anconeal process is directed to the medial part, the trochlear notch having the appearance of a semicircle. Below the radial incision there is an elongated fossa, delimited by an evident ridge.

The acropodium consists of five fingers, the first finger being the smallest.

Synthetically, it can be concluded that the establishment of the species belonging to the *Macropus rufogriseus* is easily made according to the characters of the scapula and the long bones. However, the bones of the autopodium have fewer specific elements that could be observed by us, which is why we recommend a further study of the bones of autopodium.

References

1. **Barone, R.,** Anatomie comparée des mammiferes domestiques, Tome premier - Osteologie, Imprimerie des Beaux – Arts s.a. - J. Tixier & fils, Lyon,1966.
2. **Bensley B.A.,** On the evolution of the Australian Marsupialia with remarks on the relationships of the marsupials in general,Transactions of the Linnean Society of London, 2nd Series, Zoology, 1903, 9, 3, 83-217.
3. **Boardman, W.,** On the anatomy and functional adapta-tionof the thorax and pectoral girdle in the wallaroo (*Macropusrobus-tus*), Proceedings of the Linnean Society of New South Wales, 1941, 66, 349-38.
4. **Collins, L.R.,** Monotremes and marsupials. A reference for zoological institutions, Smithsonian Institution Press, 1973.
5. **Dawson, L., Flannery, T.,** Taxonomic and phylogenetic status of living and fossil kangaroos and wallabies of the genus *Macropus* Shaw (Macropodidae:

Marsupialia), with a new subgeneric name for the larger wallabies, Australian Journal of Zoology, 1985, 33, 473-498.
6. **Dawson, R., Milne, N., Warburton, N.,** Muscular anatomy of the tail of the western grey kangaroo, *Macropus fuliginosus,* Australian Journal of Zoology, 2014, 62, 2, 166-174.
7. **Georgescu, B., Predoi, G., Roşu, Petronela Mihaela, Dumitrescu, I., Belu, C.,** Anatomia comparată a vertebratelor, Ed. Ceres, Bucureşti, 2017.
8. **Getty, R.,** The anatomy of the domestic animals, fifth edition, Saunders Company, Philadelphia, London, Toronto, 1975.
9. **Hopwood, P.R., Butterfield R.M.,** The Locomotor Apparatus of the Crus and Pes of the Eastern Gray Kangaroo, Macropus-Giganteus, Australian Journal of Zoology, 1990, 38, 4, 397-413.
10. **Harvey, K.J., Warburton, N.,** Forelimb musculature of kangaroos with particular emphasis on the tammar wallaby *Macropus eugenii* (Desmarest, 1817), Australian Mammalogy, 2010, 32, 1, 1-9.
11. **König, H.E., Liebich, H.G.,** Veterinary Anatomy of Domestic Mammals. Schattauer GmbH, Stuttgart, New York, 2014.
12. **Roşu, P.M., Georgescu, B., Raita, Ş.M., Belu, C., Purdoiu, L., Predoi, G.,** Morphological features of the skull in the dwarf kangaroo (*Macropus rufogriseus*), Scientific Works. Series C. Veterinary Medicine, 2021, LXVII, 2, 19-22.
13. **Shoaib, M.B.,** Some Anatomical Studies on the Muscles of the Antebrachium and Manus Regions of Eastern Grey Kangaroo (*Macropus giganteus*), Journal of Veterinary Anatomy, 2009, 2, 1, 1-15.
14. **Saber, A.S., Basma, M. K.,** Some Morphological and Radiological Studies on the Pelvic Limb Skeleton of Lumholtz Tree-Kangaroo (*Dendrolagus lumholtzii*), Journal of Veterinary Anatomy, 2019, 2, 12, 1, 19-34.
15. **Windle, B.C.A., Parsons, F.G.,** On the Anatomy of *Macropus Rufus*, Journal Anatomy Physiology, 1897, 32, 1, 119-134.
16. ***Nomina Anatomica Veterinaria, Fifth edition, Published by the Editorial Committee Hannover (Germany), Ghent (Belgium), Columbia, MO (U.S.A.), Rio de Janeiro (Brazil), 2017.

THE EFFECTS OF DIETARY SUPPLEMENTATION WITH PHYTOADDITIVES ON THE PRODUCTIVE PERFORMANCES OF WEANED PIGLETS

SÎRBU C.B., HOTEA I., TÎRZIU E., HERMAN V., PLOTUNA A.M., SÎRBU B.A.M., COLIBAR O.

Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645, Calea Aradului No. 119, Timisoara, Romania
E-mail: ionelahotea@gmail.com

Summary

Since the European Union banned the use of antibiotics in animal nutrition, natural alternatives have been sought for the control of various pathologies. This led to the concept of phytoadditives, and these natural plant extracts (phytoadditives) are gaining ground in pig breeding. In the present study, phytoadditives were tested in order to improve growth indicators in piglets, by reducing the incidence of digestive pathologies in animals and improving food indices with an effect on increasing productive parameters. Thus, 36 piglets, divided into 3 experimental groups (White, Green and Blue) received a herbal animal feed supplement for 28 days. At the end of the experiment, the productive parameters were evaluated: average daily gaining (ADG), specific consumption and feed conversion rate (FCR), observing the lowest values in the Blue group, for ADG and specific consumption, instead, at the same group, obtaining the best values for FCR. For the White and Green groups, average values were obtained. This study highlights the effectiveness of using phytoadditives in piglet feed.

Keywords: phytoadditives, piglets nutrition, productive parameters.

Human society today is going through a particularly complex phase, characterised by a demographic 'explosion' and a continuous rise in living standards. In this context, human nutrition takes on new dimensions and is urgently needed:

- increasing the quantity and quality of food products;
- their equitable distribution, both overall and zonally;
- use of all food resources;
- finding new assortments with increased nutrient, particularly protein content (2,14).

With the increasing demands of the population for pork and the increasing number of pigs, various pathologies involving the use of antibiotics have emerged. Now the European Food Safety Agency and the European Centre for Disease Prevention and Control have published the latest report on increasing resistance to widely used antibiotics (3, 4, 20, 21).

In this context, the use of plant genetic products in pig feed is promoted as a possible prophylactic alternative against gastrointestinal diseases. These phytogenetic products can be used as growth promoters in pig nutrition (17). Feed

additives are not nutrients and are not needed in animal diets (9). However, they often lead to improved stocking rates, feed conversions and are generally profitable when managed correctly. Some feed additives are regulated by the US Food and Drug Administration (FDA) and it is essential that regulations on their use and level of inclusion are closely followed. The regulations are published annually in the Feed Additives Regulation. Adequate information on use and withdrawal (if necessary) for each additive can be obtained from the manufacturer and/or from the label affixed to commercial feeding stuffs (12, 15).

This work was supported by the contract of the Nutrition discipline: "Prototype product test with weaned piglets" part of the „Research aimed at developing a prototype of herbal animal feed supplement for pigs to reduce antibiotics usage, environmental load and achieving higher meat quality", project no.m2017-1.3.1 –VKE -2017-00001.

The aim of this experiment was to test in the feed of weaned piglets a plant feed supplement in order to reduce the use of antibiotics, the impact on growth indicators in piglets, by reducing the incidence of digestive pathologies among animals and improving food indices with an effect on increasing productive parameters.

Materials and methods

The experiment was carried out on a semi-intensive pig farm in Timis County. The duration of the experiment was 28 days and was performed in accordance with applicable law.

The present study was carried out on 36 hybrid piglets (Large White, Duroc and Pietrain breeds) separated from their dams after weaning. The piglets were distributed in three groups. Each group of 12 piglets was divided into three identical groups of 4 individuals each. To each group was given a colour code as follows: the White Lot, the Blue Lot and the Green Lot (Fig. 1).

The piglets were identified by being assigned a number from the national animal register and ear-tagged according to the regulations in force by the concession veterinarian. The shelters were partitioned to have separate spaces for each group of 4 piglets. Each box was equipped with feeders and waterers.

The feed bags, stalls and each piglet were marked with distinct colour: white, blue and green for a better conduct of the experiment. The feed given consisted of cereal mixtures of very good quality, corresponding to the species and age category. The remaining unaccounted fodder and leftovers were weighed daily.

All the activities carried out were aimed at comparing the productive performance of the test batches compared to the control batch.

The animals came from the farm where the experiment was carried out and returned to the normal farm circuit at the end in good condition.

The following parameters have been established:

- initial weight of each piglet by individual weighing;

- final weight of each piglet by individual weighing (Fig. 2);
- daily feed consumption (without waste) for each group of piglets (kg);
- evolution of the health status of the piglets;
- feed consumption was correlated with body mass gain and on this basis the feed conversion ratio (FCR in kg/kg) was calculated.

These data allowed to calculate and follow the evolution of body mass, daily intake, average body weight gain and feed conversion ratio for all animals.

Fig. 1. Piglet lotting

Fig. 2. Weighing piglets

Results and discussions

The weaned piglets were allocated to the boxes by a randomized process, but trying to maintain the balance between males and females in each group.

At the beginning and end of the experiment, the piglets went through a period of adaptation to the new diet. The new feed has been gradually introduced into the piglet's diet in increasing amounts to allow the digestive tract to adjust. At the same time, old feed was gradually reduced until it was completely replaced by new feed. This was followed by a period of 28 days during which the piglets were fed differentially with the three types of feed conventionally marked white, blue and green. Each type of feed was given to 12 piglets divided into groups of 4. After completion of the 28-day feeding period on the same feed, the animals were weighed again and this value was recorded as the final individual weight, showing a significant increase in body mass. Initial and final weighing was done individually.

Based on the differences between the final and initial weights, the increase in body mass was found. From the data obtained, the average daily gain (ADG) was calculated and the comparative results are shown in Table 1 and Fig. 3.

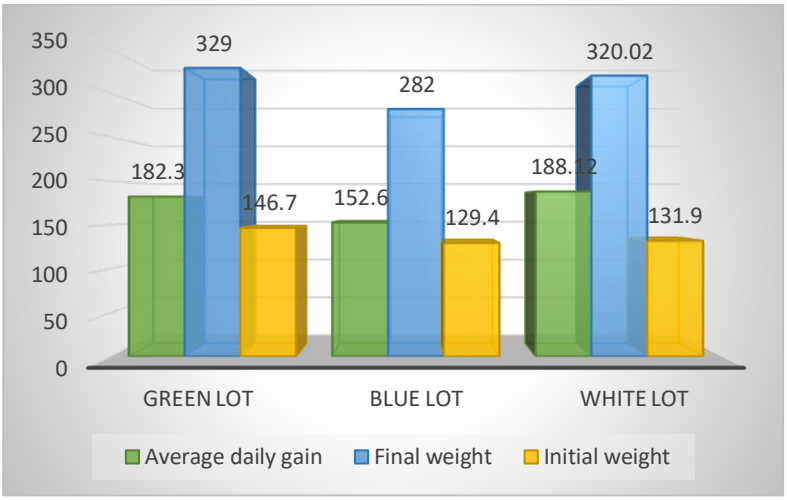


Fig. 3. Comparison of initial and final weights and average daily gain in piglets

From the analysis of the data presented, it can be seen that the lot with the highest body weight is the Green lot (329 kg) compared to the other lots (282 kg for the Blue lot and 320.02 kg for the White lot).

The average daily gain of piglets is relatively close between the White and Green lots (559 grams and 542 grams respectively). The Blue lot had the lowest average daily gain (454 grams).

Daily feed consumption was monitored daily. The total amount of feed ingested for each lot of piglets throughout the experimental period was recorded individually for each piglet, and averaged for each lot at the end.

Table 1

Difference between final weight and initial weight at the end of the experiment (average daily gain – ADG)

Lot	Group	ADG (kg)	Lot	Group	ADG (kg)	Lot	Group	ADG (kg)	Total ADG
WHITE	I	17.64	BLUE	IV	10.08	GREEN	VII	14.48	523.02
		12.60			5.32			15.68	
		15.12			8.96			14.84	
		13.72			8.40			17.64	
	II	16.80		V	12.04		VIII	15.50	
		15.68			14.00			15.12	
		12.88			18.48			12.88	
		14.84			17.64			12.60	
	III	12.88		VI	12.04		IX	16.80	
		12.60			15.68			12.32	
		26.56			11.48			18.76	
		16.80			18.48			15.68	
Total ADG – WHITE lot		188.12	Total ADG – BLUE lot		152.60	Total ADG – GREEN lot		182.30	

It is found that the lowest amount of feed ingested (379 kg) is observed in the Blue lot, which also had a lower body weight. The other lots were significantly close (402 kg for the White lot and 403 kg for the Green lot). These data were used to calculate feed use efficiency (Table 2).

All the results presented were interpreted by calculating food use efficiency (1). Taking into account feed consumption and body weight gain, the Feed Conversion Ratio (FCR) was calculated. This value is the result of the ratio of feed intake to body mass gain.

Table 2

Total piglet feed consumption, ADG and feed conversion ratio (FCR)

Lot	Amount of feed ingested (kg)	Total ADG (kg)	FCR Amount of feed ingested/ total ADG (kg/ kg)
WHITE	402	188.12	2.13
BLUE	379	152.60	2.48
GREEN	403	182.30	2.21

It is found that for each kilogram of body weight the piglets consumed a variable amount of feed. The lowest value was recorded for the White lot (2.13kg), then for the Green lot (2.21kg) and the highest value was recorded for the Blue lot (2.48kg) (Fig. 4). The lower the FCR value, the better the ration utilization was. So, the least efficient utilization of the feed was the Blue lot, while the other lots recorded close values.

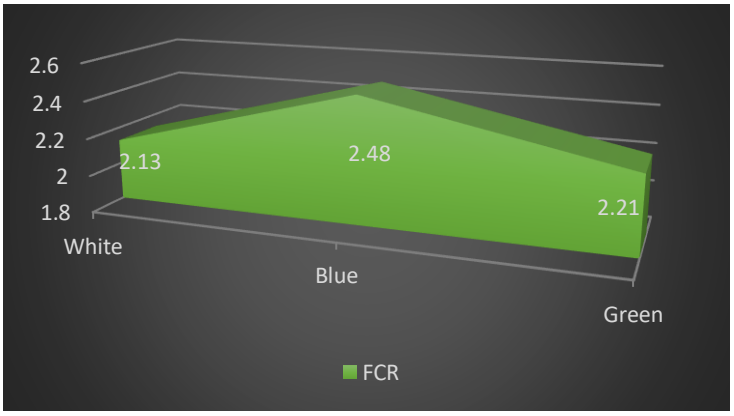


Fig. 4. Feed conversion ratio

The data obtained were compared with other studies published in the literature and it was found that, in general, the results are similar.

Zhang et al. (19) administered a feed with the aim of improving piglet health and intestinal development. The study was conducted over a 28-day period and found an improvement in the feed conversion rate of piglets, reduced diarrhea rate in weaned piglets, also found enhanced antioxidant activity and improved intestinal microbial flora (11).

The main objective of this study conducted by Flutura et al. (8) in Albania was to investigate the effects of pellets and meal feed on performance parameters: body weight, average daily gain, feed conversion ratio (FCR) and apparent nutrient

digestibility of weaned piglets. Forty weaned piglets were divided into two groups (control and experimental group) for 6 weeks. The use of pellets and meal feed in the piglets' feed improved growth performance: body weight 2.5%, daily weight gain 2.7%, feed intake 1.1% and feed conversion ratio 1.6% more than the control group for the whole experimental period. Fibre digestibility was slightly increased and fat digestibility was slightly decreased. Overall, a positive effect of pelleted feed on the growth performance of piglets was found (18).

Also, Festim et al. (7) studied the effectiveness of combining two feed additives to enhance the growth performance of piglets. The effects on the growth of 30 piglets were studied for six weeks. At the end of the experimental period the combined feed additive supplementation slightly improved daily weight gain by 2.7% and feed conversion ratio (FCR) by 4.4% (10).

In the study by Dell`Anno et al. (6) the effect of leonardite (supplement) was studied on 120 piglets, Great White and Landrace hybrids, for 40 days. Body weight, average daily feed intake (ADFI), feed conversion ratio (FCR) were measured during the experimental period. Body weight was recorded individually using a validated scale at baseline. Feed intake was recorded weekly for each feeder. Based on the body weight results, average daily gain and feed conversion ratio (FCR) were calculated. Body weight increased from 8.71 on day 0 to 20.17 on day 40 and FCR was 1.77 on days 0 – 14 and 2.09 on days 28 – 40 (16).

In 2019, Son Hoang Nghia et al. (13) conducted a study to evaluate the effect of feed supplementation with mineral nanoparticles on piglet growth by evaluating average daily gain, total feed intake and feed conversion ratio. A total of 240 newly weaned Duroc, Landrace and Yorkshire hybrid piglets were grouped into 4 lots. Piglets in group 1 were fed diets supplemented with 1.6 mg/kg nMn; 20 mg/kg nFe; 2 mg/kg nCu; 0.1 mg/kg Co; 0.1 mg/kg nSe and 20 mg/kg nZn. The nano-mineral content of lots II, III and IV were 2, 4 and 8 times higher than the amount of nano-minerals fed to lot I. The piglets in lot IV had the highest live weight (36.68 ± 0.34 kg) compared to the other lots. All 4 experimental lots showed lower feed conversion ratio compared to the control lot. No significant difference was observed between the feed conversion ratio of piglets in lot I (2.85 ± 0.08), lot II (2.69 ± 0.13), lot III (2.73 ± 0.03), and lot IV (2.69 ± 0.07) (5).

Conclusions

The average daily gain of piglets is relatively close between the White and Green lots (559 grams and 542 grams respectively). The Blue lot had the lowest average daily gain.

The lowest amount of feed ingested was in the Blue lot, which also had the lowest body weight.

The lowest (and best) FCR value was recorded in the White lot (2.13), then in the Green lot (2.21) and the highest value was observed in the Blue lot (2.48).

References

1. **AOAC, A.,** Official methods of analysis, Washington, DC, USA. AOAC International, 2006.
2. **Blair, R.,** Nutrition and Feeding of Organic Pigs 2nd Edition, CABI, 2018.
3. **Brooks, P.H., Beal, J.D., Dmeckova, V., Niven, S.,** Role of probiotics in animal nutrition and their link to the demands of European consumers, ID-Lelystad, 2003.
4. **Cromwell, G.L.,** Nutritional Requirements of Pigs, Merck Veterinary Manual, 2015.
5. **de Meer, K., Smolders, H.C., Meesterburrie, J., de Sain-van der Velden, M., Voorbij, H.A., Okken, A., Reijngoud, D., Kulik, W.,** A single food bolus stimulates albumin synthesis in growing piglets, Journal of pediatric gastroenterology and nutrition, 2000, 31, 3, 251-257.
6. **Dell'anno, M., Hejna, M., Sotira, S., Caprarulo, V., Reggi, S., Pilu, R., Miragoli, F., Callegari, M.L., Panseri, S., Rossi. L.,** Evaluation of leonardite as a feed additive on lipid metabolism and growth of weaned piglets, Animal Feed Science and Technology, 2020, 266, 114519.
7. **Festim, S., Etleva, D.,** Using feed additives as a way to improve growth performance in weaned piglets, Albanian Journal of Agricultural Sciences, 2013, 12, 4, 725-728.
8. **Flutura, S., Etleva, D.,** The effect of pellet feed on the performance and nutrient's digestibility of weaned piglets, Albanian Journal of Agricultural Sciences, 2012, 4, 11, 275-278.
9. **Fuller, R.,** Probiotics for farm animals, Probiotics: A critical Review, Horizon Scientific Press, 1999.
10. **Komar, A.H.A-J., Yaser, K.D.,** Effect of Different Feed Additives on Growth Performance and Production in Livestock, International Journal of Agriculture and Forestry, 2019, 9, 1, 16-31.
11. **Liu, P., Zhao, J., Wang, W., Guo, P., Lu, W., Wang, C., Johnston, L.J., Zhao, Y., Wu, X., Xu, C., Zhang, J., Ma, X.,** Dietary corn bran altered the diversity of microbial communities and cytokine production in weaned pigs, Frontiers in Microbiology, 2018, 9, 2090.
12. **Miller, E.R., Ullrey, D.E., Lewis, A.J.,** Swine Nutrition, Elsevier Inc., 1991.
13. **Nghia, S.H., Le Huy, T.N., Thi Mai, T.B., Trung, C.D., Le Thanh, L.,** Effects of Supplementation of Mineral Nano Particles on Weaned Piglet Growth, International Journal of Horticulture, Agriculture and Food science, 2019, 3, 2, 37-40.
14. **Păsărin, B.,** Tehnologia creșterii suinelor, Ediția a II-a, Ed. Ion Ionescu de la Brad, Iași, 2007.
15. **Southern, L.L., Adeola, O., De Lange, C.F.M., Hill, G.M., Lindemann, M.D., Miller, P.S., Odle, J., Stein, H.H., Trottier, H.L.,** Nutrient requirements of swine, The National Academies Press., 2012.

16. **Trckova, M., Lorencova, A., Babak, V., Neca, J., & Ciganek, M.,** The effect of leonardite and lignite on the health of weaned piglets, Research in Veterinary Science, 2018, 119, 134-142.
17. **Veizaj-Delia, E., Piu, Th., Lekaj, P., Tafaj, M.,** Using combined probiotic to improve growth performance of weaned piglets on extensive farm conditions, Livestock Science, 2010, 134, 1-3, 249-251.
18. **Veizaj, E., Tafaj, M., Männer, K.,** The effect of the combined probiotic preparation on growth performance, digestibility and microbial composition of faeces of weaned piglets, Agricultura, 2008, 5, 37-41.
19. **Zhang, W., Bao, C., Wang, J., Zang, J., Cao, Y.,** Administration of *Saccharomyces boulardii* mafic-1701 improves feed conversion ratio, promotes antioxidant capacity, alleviates intestinal inflammation and modulates gut microbiota in weaned piglets, Journal of Animal Science and Biotechnology, 2020, 11, 1, 112.
20. ***** EFSA,** Guidance on the assessment of the efficacy of feed additives, EFSA Journal, 2018, 16, 5, 5274, https://doi.org/10.2903/j.efsa.2018.5274.
21. ***** Fayuan, G.,** Application of Yeast Products for Antibiotic – free Diets, 2021, https://en.angelyeast.com/blog/animal-nutrition/yeast-products-for-antibiotic-free-diets.html.

ANTIMICROBIAL RESISTANCE OF *STAPHYLOCOCCUS SPP*. IN BOVINE MASTITIS

SPĂTARU I.I., TORDA I., MIRCU C.

Banat University of Agricultural Sciences and Veterinary Medicine Timișoara,
Faculty of Veterinary Medicine, 300645,
Calea Aradului, No. 119, Timișoara, România
E-mail: irinaa.spataru@gmail.com

Summary

Bovine mastitis is one of the most important bacterial diseases of dairy cattle worldwide and is responsible for huge economic losses for the dairy industry and milk producers. Bovine intramammary infection caused by *Staphylococcus aureus* is characterized by subclinical mastitis and persistent long-term infection, and the curative effect of antimicrobial therapy is low. Methicillin-resistant *St. aureus* (MRSA) has become a notable problem as a causative agent in diseases of domestic animals, including bovine mastitis, and most cases of this disease are caused by *S. aureus* ST398. Moreover, bovine mastitis caused by *MRSA*, well known as a typical multidrug-resistant organism, has been reported in all parts of the world and has attracted a great deal of attention. Staphylococcal resistance to methicillin and all *β-lactam* antibiotics is associated with the presence of the *mecA* gene encoding *PBF2a*, an alternative penicillin-binding protein. Thus, the *mecA* gene is responsible for staphylococcal resistance to penicillin-like antibiotics. In order to survive, microorganisms undergo certain mutations in the chromosomal DNA or RNA, providing them resistance. The cell wall of bacteria acts as a barrier and helps them survive, but due to altered chromosomal DNA or genetic mutations, the composition of the cell wall or plasma membrane changes, thus contributing to the phenomenon of resistance. This study aims to investigate the antimicrobial resistance (AMR) profiles of mastitis-causing *S. aureus*.

Key words: mastitis, AMR, *Staphylococcus aureus,* β-lactam antibiotics.

Bovine mastitis is an inflammatory response of the mammary gland tissue that can be caused by infections with microorganisms or physical trauma. It is considered one of the most common diseases that can cause economic losses in the dairy industry due to low milk production and quality (8).

Depending on the degree of inflammation, mastitis can be of three types: clinical, subclinical and chronic. Clinical mastitis is obvious and easily identified by visible signs, such as fever, inflammation of the mammary gland, loss of appetite and more severe cases can even be fatal (17).

Unlike clinical mastitis, subclinical mastitis does not cause visible signs of the udder or milk, but milk production decreases with increasing somatic cell counts. Chronic mastitis is an inflammatory process that lasts for several months and occurs at irregular intervals (9).

Risk factors

Intramammary bacterial infection is considered to be the main cause of bovine mastitis. Depending on the bacterial origin, mastitis can be classified as: contagious and environmental. Contagious mastitis can be transmitted from one female to another, especially during milking (11).

Pathogens such as *Staphylococcus aureus*, *Streptococcus agalactiae* and less common species such as *Mycoplasma bovis* and *Corynebacterium* survive on both the skin of the mammary gland and the nipple, colonizing the canal. These pathogens are able to cause subclinical infections accompanied by increases in the level of somatic cells (17).

Environmental pathogens are described as opportunistic microorganisms that seek the opportunity to cause an infection. Being found in shelters and on bedding, these bacteria enter the mammary glands through the nipple canal where they colonize, proliferate and then release toxins, thus affecting the cells of the mammary gland (6, 12).

An increase in lactate dehydrogenase (LDH) activity in milk is an indication of infection. Inflammatory responses are caused immediately after infection (9). Somatic cell counts and inflammatory cytokine levels, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and (IL)-8 are elevated (14).

S.aureus is the most common gram-positive pathogen and is associated with various forms of clinical and subclinical mastitis. Therefore, infection with this pathogen is always milder causing chronic mastitis that can last for several months. *S. aureus* infections are treated with antibiotics (7).

However, some authors have shown that antibiotic therapy is not a very effective method due to the resistance developed by *S. aureus* strains to *β-lactam* antibiotics, especially methicillin, which is also known as *MRSA* (Staphylococcus aureus methicillin-resistant) (13).

Studies have shown that *MRSA* poses a threat to both humans and animals. This pathogen is often found in cow's milk and has been clinically proven to be a cause of mastitis. In addition, infections caused by *MRSA* have been associated with high economic losses and increased morbidity (19).

Over time, antibiotic therapy has become one of the most widely used practices for controlling bovine mastitis. Some antibiotics such as tetracycline, chloramphenicol, ciprofloxacin, novobicin and vancomycin have been reported to be poorly effective against *S. aureus*. Improper use of antimicrobial agents in veterinary practice is a major factor in the spread of antibiotic-resistant pathogens in human populations, so it can lead to public health problems (4, 8).

Cells of host innate immunity

When microorganisms cross the epithelial barrier and begin to replicate in the host tissues, they are immediately recognized, then ingested and disintegrated by mononuclear phagocytes or macrophages residing in the tissues. Neutrophils,

another important family of phagocytes are short-lived cells, present in abundance in the blood but not in the tissues. Macrophages and neutrophils play a key role in innate immunity, as they can effectively destroy many pathogens without the help of adaptive immunity. In particular, neutrophils are central players in the interaction between the host and *S. aureus* (18).

During infection, neutrophils leave the bloodstream and migrate to the site of infection in a multi-stage process, mediated by adhesive interactions that are regulated by cytokines and chemokines (2).

Cytokines are small proteins released by various cells in the body in response to an activating stimulus and which induce responses by binding to specific receptors. They can act in an autocrine or paracrine manner. Chemokines are a class of cytokines that have chemoattractant properties, inducing cells with the appropriate receptors to migrate to the source of the chemokine. Chemokines mainly recruit leukocytes, especially monocytes and neutrophils, and other blood effector cells to sites of infection (2).

Mechanisms of antibiotic resistance in *Staphylococcocus aureus*

Staphylococcal resistance to methicillin and all *β-lactam* antibiotics is associated with the presence of the *mecA* gene encoding *PBF2a*, an alternative penicillin-binding protein. Thus, the *mecA* gene is responsible for staphylococcal resistance to penicillin-like antibiotics. Both the mecA gene and other functional genes are located on a mobile genetic element called the mec chromosomal staphylococcal cassette (SCCmec) (15).

Zong et al. (20) showed that SCCmec contains two essential components: the *Mec* gene complex and the *Ccr* gene complex. The Mec gene complex is represented by the *mecA* gene, regulatory genes and associated insertion sequences. In addition, it contains plasmids and transposons and is divided into six different classes: A, B, C1, C2, D and E (20).

The *Ccr* gene complex comprises one or more *Ccr* genes, which have the ability to encode recombinases that mediate the integration and removal of SCCmec into and from the chromosome.

In order to survive, microorganisms undergo certain mutations in the chromosomal DNA or RNA which gives them resistance. The cell wall of bacteria acts as a barrier and helps them survive, but due to altered chromosomal DNA or genetic mutations, the composition of the cell wall or plasma membrane changes, thus contributing to the phenomenon of resistance (20).

SCCmec consists of two components: the Mec gene complex with a role in encoding the methicillin resistance gene (mecA) and two other regulatory genes (mecR1, mec1) and the Ccr gene complex that deals with the movement of the entire SCC complex (Fig. 1).

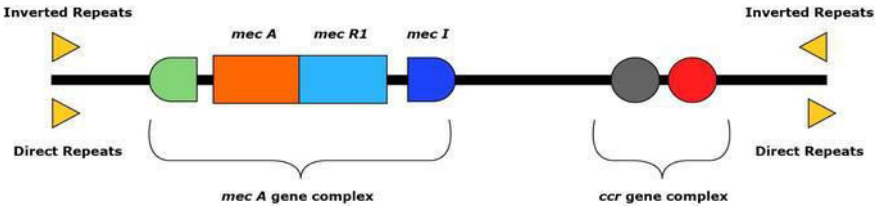


Fig. 1. SCCmec structure

Alternative treatments for bovine mastitis

Also it was demonstrated that Yi-Xiong-Tang (YXT) extract from the two plants, *Angelica dahurica* and *Rheum officinale* has beneficial effects in the treatment of mastitis. The results showed that the levels of inflammation indicators (cytokines, TNF-α, IL-6 and IL-8) in milk returned to normal after treatment, suggesting that YXT had anti-inflammatory and immunomodulatory activities. In addition, the authors argue that YXT treatment requires shorter treatment compared to antibiotic therapy and is less likely to cause drug resistance to develop causative agents (5, 14).

Some authors report that the treatment of bovine mastitis can be performed by oral administration of plant decoctions with similar properties to *A. dahurica* and *R.officinale*, such as *Taxaracum mongolicum*, *Lonicera japonica*, *Pericarpium Citri Reticulatae Viride* and *Glycyrrhiza uralensis*, but due to large body weight of cows requires large amounts of plants for each dose. Also, the preparation of such a decoction and oral administration to mastitis cows is costly and time consuming (11).

Other authors report the antibacterial effects against staphylococcal strains of an ethanolic leaf extract of *Rhodomyrtus tomentosa*, a traditional plant of the Tai plant, and of Rhodomyrtone, a purified compound from this plant. This extract has been shown to have a strong *S. aureus* inhibitory effect similar to that obtained with vancomycin, an important anti-*S. aureus* medicine in human medicine (16).

A study in northwestern Pakistan highlighted the effectiveness of the plants *Allium sativum*, *Bunium persicum*, *Oryza sativa* and *Triticum aestivum* against pathogens such as *S. aureus*, *E.coli* and *K. pneumoniae* that cause mastitis. The plants were phytochemically tested for alkaloids, flavonoids and saponins and all extracts were found to significantly inhibit the activity of the bacterial strains examined (3).

Conclusions

Staphylococcus aureus is the main pathogen that causes bovine mastitis, compromising animal health and economy.

The widespread use of antibiotics on cattle farms and the development of bacterial resistance to traditional antibiotics have led to the search for alternatives to antibiotics for the treatment of bovine mastitis.

Because many medicinal plants have traditionally been used to treat infectious diseases, plant extracts are an interesting source of new antibacterial substances.

References

1. **Abrahms, M., Persson, Y., Kanyima, B.M., Bage, R.,** Prevalence of subclinical mastitis in dairy farms in urban and peri-urban areas of Kampala, Uganda, Tropical Animal Health and Production, 2014, 46, 99-105.
2. **Allen, S.J., Crown, S.E., Handel, T.M.,** Chemokine: receptor structure, interactions and antagonism, Annual Review of Immunology, 2007, 25, 787-820.
3. **Amber, R., Adnan, M., Tariq, A., Khan, S.N., Mussarat, S., Hashem, A., Al-Huail, A.A., Al-Arjani, A.F.,** Antibacterial activity of selected medicinal plants of northwest Pakistan traditionally used against Mastitis in livestock, Saudi Journal of Biological Sciences, 2018, 25, 154-161.
4. **Anderson, K., Azizoglu, R.,** Detection and Causes of Bovine Mastitis with Emphasis on Staphylococcus aureus, Encyclopedia of Agriculture and Food Systems, 2014, 2, 435-440.
5. **Bannerman, D.D.,** Pathogen-dependent induction of cytokines and other soluble inflammatory mediators during intramammary infection of dairy cows, Journal of Animal Science, 2009, 87, 10-25.
6. **Bradley, A.J.,** Bovine mastitis: an evolving disease, Veterinary Journal, 2002, 164, 116-28.
7. **Gilbert, F.B., Cunha, P., Jensen, K., Glass, E.J., Foucras, G., Robert-Granié, C., Rupp, R., Rainard, P.,** Differential response of bovine mammary epithelial cells to *Staphylococcus aureus* or *Escherichia coli* agonists of the innate immune system, Veterinary Research, 2013, 44, 40.
8. **Gomes, F., Henriques, M.,** Control of Bovine Mastitis: Old and Recent Therapeutic Approaches, Current Microbiology, 2015, 72, 377-382.
9. **Kalantari, A., Safi, S., Foroushani, A.,** Milk lactate dehydrogenase and alkaline phosphatase as biomarkers in detection of bovine subclinical mastitis, Annals of Biological Research, 2013, 4, 2, 302-307.
10. **Khan, M., Khan, A.,** Basic facts of mastitis in dairy animals: a review, Pakistan Veterinary Journal, 2006, 26, 204-208.

11. **Lakew, B.T., Fayera, T., Ali, Y.M.,** Risk factors for bovine mastitis with the isolation and identification of *Streptococcus agalactiae* from farms in and around Haramaya district, eastern Ethiopia, Tropical Animal Health and Production, 2019, 51, 1507-13.
12. **Nikaido, H.,** Multidrug resistance in bacteria, Annual Review of Biochemistry, 2009, 78, 119-46.
13. **Oliveira, M., Bexiga, R., Nunes, S.F., Vilela, C.L.,** Invasive potential of biofilm-forming *Staphylococci* bovine subclinical mastitis isolates, Journal Veterinary Science, 2011, 12, 95-7.
14. **Persson, W.K., Colditz, I.G., Lun, S., Östensson, K.,** Cytokines in mammary lymph and milk during endotoxin-induced bovine mastitis, Research in Veterinary Science, 2003, 74, 31-36.
15. **Petinaki, E.,** Detection of *mec*A, *mec*R1 and *mec*I genes among clinical isolates of methicillin-resistant staphylococci by combined polymerase chain reactions. Journal of Antimicrobial Chemotherapy, 2001, 47, 297-304.
16. **Saising, J., Hiranrat, A., Mahabusarakam, W., Ongsakul, M., Voravuthikunchai, S.P.,** Rhodomyrtone from Rhodomyrtus tomentosa (Aiton) Hassk as a natural antibiotic for staphylococcal cutaneous infections, Health Science Journal, 2008, 54, 589-595.
17. **Schreiner, D., Ruegg, P.,** Effects of tail docking on milk quality and cow cleanliness, Journal of Dairy Science, 2002, 85, 2503-11.
18. **Spaan, A.N., Surewaard, B.G., Nijland, R., van Strij, P.J.A.,** Neutrophils versus *Staphylococcus aureus*: a biological tug of war, Annual Review of Microbiology, 2013, 67, 629-650.
19. **Witte, W., Strommenger, B., Stanek, C., Cuny, C.,** Methicillin-resistant Staphylococcus aureus ST398 in Humans and Animals, Central Europe. Emerging Infectious Disease, 2007, 13, 255-258.
20. **Zong, Z., Peng, C., Lü, X.,** Diversity of SCC*mec* elements in methicillin-resistant coagulase-negative staphylococci clinical isolates, PLoS One, 2011, 6, 5, e20191.

STUDY ON THE PREVALENCE OF DEMODICOSIS IN TWO DOG SHELTERS IN MEHEDINȚI COUNTY

NEGRESCU A.[1], MEDERLE N.[1], MORARIU S.[1], MARIN A.M.[1], SAVICI J.[1], ALBU KAYA M.G.[2], DĂRĂBUȘ Gh.[1]

[1]Banat's University of Agricultural Sciences and Veterinary Medicine" King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645, Calea Aradului No.119, Timisoara, Romania
[2]Natl Res & Dev Inst Text & Leather, Div Leather & Footwear Res Inst, Dept Collagen, Bucharest 031215, Romania
E-mail: negrescu.adina-mh@ansvsa.ro

Summary

Demodicosis produced by species of the genus *Demodex* (*D. canis, D. injai, D. cornei*) is one of the most important skin diseases diagnosed in dogs. The study was carried out in the period 2019-2021, at dogs (520 animals) from the Strehaia and Turnu Severin shelters, dogs of different breeds, both sexes and different ages. From the total number of dogs present in the shelter over a period of three years, demodicosis was diagnosed with a prevalence of 22.88% (119/ 520). Mixed-breeds aged between 3-6 months were more affected compared to the other breeds and age categories. The localized form was diagnosed in dogs in the 3-6 month category, while adult dogs showed generalized demodicosis. There are no significant correlations between the occurrence of the disease and the sex of the dogs, both sexes being equally affected. Demodicosis is and remains one of the most prevalent skin diseases of dogs, the involvement of risk factors (age, maintenance conditions) in the initiation, evolution and progress of acariosis being imminent.

Keywords: canine demodicosis, prevalence, risk factors, Mehedinti.

Demodicosis is one of the most important skin conditions diagnosed in dogs. Parasitic disease produced by species of the genus *Demodex*, demodicosis can evaluate in a dry form or can turn into a wet form when it becomes bacterially complicated. *Demodex canis* is the reference species; there are two more species responsible for the appearance of lesions, *D. injai* and *D. cornei* (5, 13, 23). Prevalence of canine demodicosis varies widely, from 0.4 to 32.78%, with risk factors clearly influencing the outbreak, clinical course and progression of parasitosis (6, 15, 17, 21, 22).

Race, gender, age, maintenance conditions, climatic conditions, associated parasitism are often cited as important intrinsic and extrinsic factors in the epidemiology of canine demodicosis (8).

The aim of the study was to identify the prevalence of demodicosis in two dog shelters from the localities of Strehaia and Turnu Severin, Mehedinți County.

Materials and methods

The study was carried out during 2019-2021, at dogs (520 animals) from the Strehaia and Turnu Severin shelters under the management of the County Council or the Territorial Support Unit (Fig. 1. a, b).

Fig. 1. a, b. Dogs shelters

The dogs included in the study were represented by: Mixed Breed (470 dogs), German Shepherd (26 dogs), Romanian Shepherd (18 dogs), Labrador (6 dogs). A total of 270 dogs were male and 250 were female. The dogs were distributed in the following age categories: 3-6 months, 180 dogs; 1-5 years, 27 dogs; 5-10 years, 306 dogs; 10-15 years, 7 dogs. (Fig. 2 – Fig. 4).

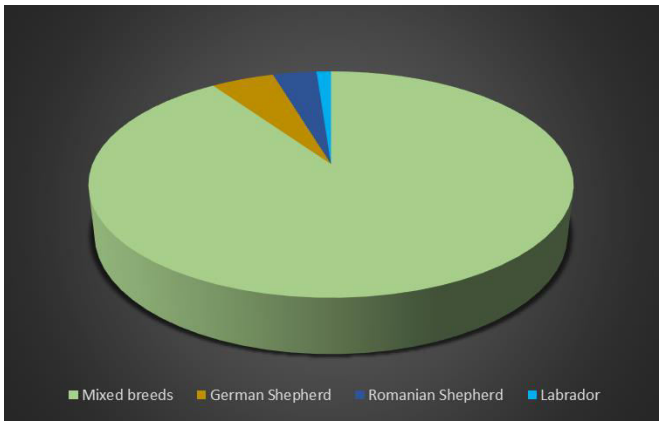


Fig. 2. Distribution of cases correlated with breed

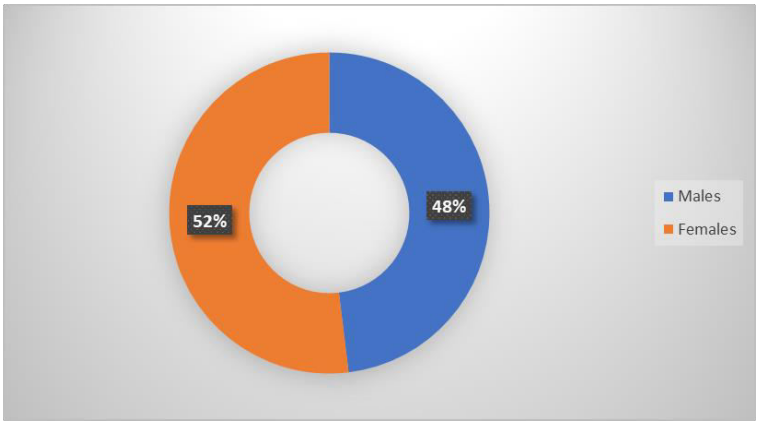


Fig. 3. Distribution of cases correlated with sex

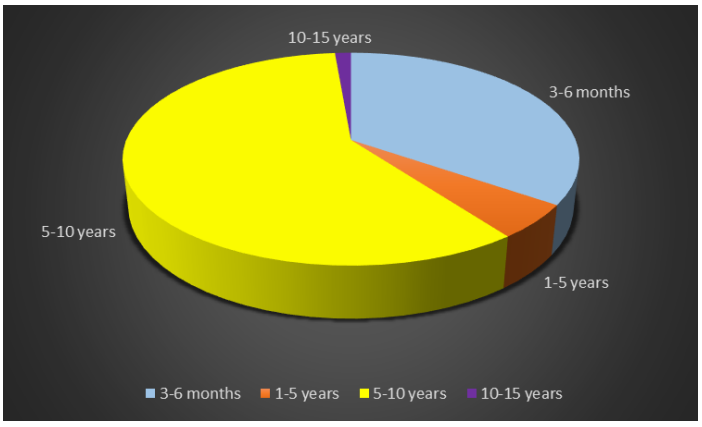


Fig. 4. Distribution of cases correlated with age

Medical Exams

To establish the diagnosis, the general clinical examination and the dermatological examination of the lesions present on the dogs' body were completed with the laboratory examinations: skin scraping, scotch tape examination, culture media for dermatophytes (DTM), bacteriological examination (24).

For the interpretation of the results and their graphic representation, we used the Excel program in which we collected all the information regarding the patients: race, sex, age, diagnosis.

Results and discussions

The clinical, dermatological examination and the microscopic examination of the skin scraping allowed the diagnosis of existing skin conditions in the two dog shelters and, implicitly, the confirmation of the presence of demodicosis (Fig. 5).

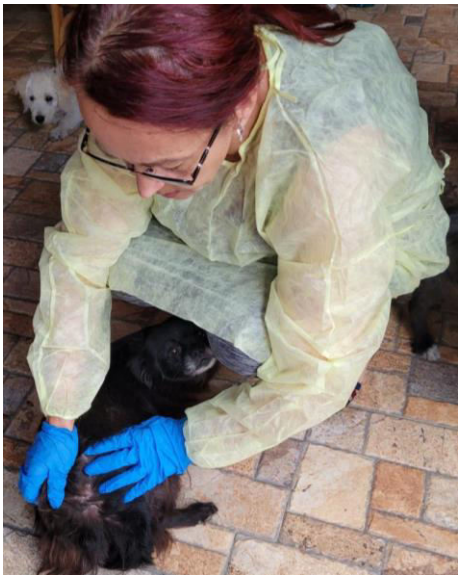


Fig. 5. Clinical examination

Demodicosis was clinically diagnosed in three forms of evolution: localized dry (erythema, well-defined alopecia areas, the presence of fine, whitish, pityriaziform scales, absence of itching; localized periocular lesions, lip commissure, cheek and ear lobe, in the ventral cervical region and on the forelimbs), generalized dry (diffuse, generalized alopecia, erythema, hyperkeratosis, hyperpigmentation, hyperseborrhea; lesions distributed on the head, trunk, limbs; general condition unchanged); pyodemodicosis (generalized alopecia, pustules, hyperpigmentation, hyperseborrhea, lichenification, crusts, ulcers, abscesses, pruritus; lesions present on the body and limbs; general condition affected) and pododemodicosis (erythema in the interdigital spaces, skin discoloration, pustules, fistulas, crusts, abscesses) (Fig. 6, Fig. 7).

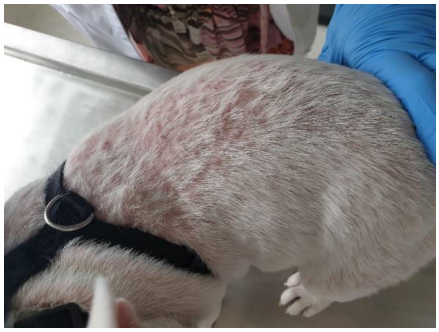


Fig. 6. Erythema

Fig. 7. Descuamation

Microscopic evidence of the mite in skin scrapings made from lesions and clarified with paraffin oil confirmed the clinical diagnosis (Fig. 8).

Fig. 8. *Demodex spp.*

From all the dogs present in the shelter over a period of three years, demodicosis was diagnosed with a prevalence of 22.88% (119 dogs/520), surpassed by allergic dermatitis to flea bites (210 dogs/520, 40.38%) and followed by sarcoptic mange (78/520, 15 %) (Fig. 9).

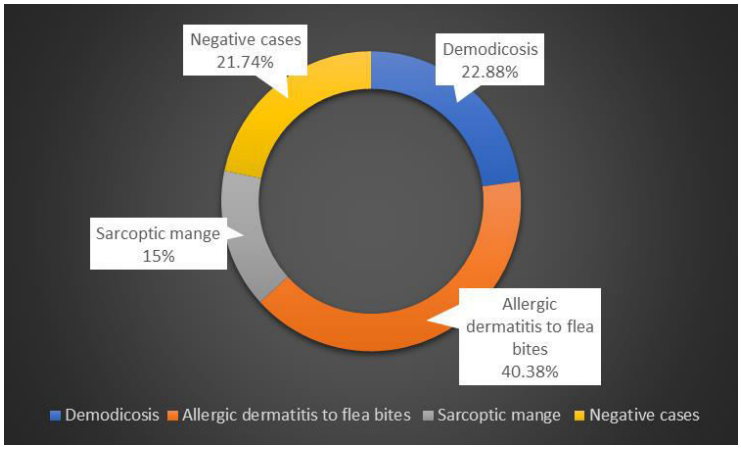


Fig. 9. The structure of skin diseases diagnosed in the dog population

Mixed-breeds aged between 3-6 months were more affected compared to the other breeds and age categories. The localized form was diagnosed in dogs in the 3-6 month category, while adult dogs showed generalized demodicosis. There are no significant correlations between the occurrence of the disease and the sex of the dogs, both sexes being equally affected.

Comparing the results obtained in the present study with those of the studies carried out in the country and abroad, we notice that values of the prevalence of canine demodicosis vary a lot, from 0.4 to 33.78%, being influenced in their dynamics by the intervention of different intrinsic and extrinsic factors (9, 16, 20).

Epidemiological studies carried out in France reveal a prevalence of demodicosis of 28.71%, followed by bacterial dermatitis (11.28%) and allergic dermatitis (10.71%); the first place in the ranking of parasitic diseases was occupied by toads (sarcoptic and otodectic 34.42%) (1). In opposition to these results, Polish researchers classify demodicosis at the top of the structure of skin diseases (48%), followed by dermatophytosis (45%) and sarcoptic mange (15%) (18), while, in Albania, parasitism with Ctenocephalides canis reached an impressive percentage of 75.7 compared to the prevalence of demodicosis (0.6%) (2, 25).

On the Asian continent, demodicosis reaches low prevalence values, from 4.9% to5.2%, parasitism with Otodectes cynotis (22%) and Sarcoptes canis (19.4%) being the most prevalent (3, 4).

In our country, studies on the prevalence of demodicosis reveal the second position in the ranking, after allergic dermatitis (19), respectively, dermatophytosis (12), with a prevalence of 32.78 and 17.77%, respectively.

The opinions that emerge from the studies carried out in the country and abroad are unanimous when correlations are made between demodicosis and the intervention of different risk factors: any breed of dog can manifest the disease,

males and females, at any age category, with localized clinical manifestations in puppies and generalized in adult dogs (14, 24).

There are, however, certain particularities related to the author and the place of the research.

Comparing the results of the present study with the results of other studies, we note that those most prone to mite infestation *Demodex*, are dogs from large breeds (German Shepherds), the age category 6 months - 1 year being the most affected and developing localized form of evolution (64.97%); the generalized form was diagnosed in adults (11.52%) (7, 10, 19).

Poor housing conditions and outdoor dogs are significant risk factors associated with the clinical expression of canine demodicosis. The results of the studies carried out by other authors join the results obtained in the study conducted on the canine population from shelters in Mehedinți County, which reveal that the most receptive to demodectic infestation are dogs living in precarious conditions (11, 20).

Conclusions

Demodicosis is and remains one of the most prevalent skin diseases of dogs, the involvement of risk factors (age, maintenance conditions) in the initiation, evolution and progress of acariosis being imminent.

Acknowledgments

This scientific paper was carried out in the Laboratory of Parasitology and Parasitic Disease at the Faculty of Veterinary Medicine Timisoara, Laboratory within the Animal Hygiene and Pathology Research Center/ BUASVM Timisoara.

References

1. **Carlotti, D.N.,** La dermatologie vétérinaire depuis vingt ans, Point Vétérinaire, 1993, 25, 155, 506-519.
2. **Carlotti, D.N., Pin, D.,** Diagnostique dermatologique, Ed. Masson, Paris, 2002.
3. **Chee, J.H., Kwon, J.K., Cho, H.S., Cho, K.O., Lee, Y.J., Abd El-Aty A.M., Shin, S.S.,** A survey of ectoparasite infestations in stray dogs of Gwang-ju City, Republic of Korea, Korean Journal Parasitology, 2008, 46, 1, 23-27.
4. **Chhabra, S., Khakra, S.S., Nauriyal, D.C.,** Prevalence of ectoparasite infestations on dogs in and around Ludhiana, Journal Research, 2000, 36, 3/4, 263-266.
5. **Desch, C.E., Hillier, A.,** *Demodex injai*: a new species of hair follicle mite (Acari: *Demodecidae*) from the domestic dog (*Canidae*), Journal Medical Entomology, 2003, 40, 2, 146-9.

6. **Fondati, A., De Lucia, M., Furiani, N., Monaco, M., Ordeix, L., Scarampella, F.,** Prevalence of *Demodex canis*-positive healthy dogs at trichoscopic examination, Veterinary Dermatology, 2010, 21, 2, 146-51.
7. **Gartner, A., Dărăbuș, G., Badea, C., Hora, F., Tilibașa, E., Mederle, N.,** Clinical diagnosis in canine demodicosis. A new approach. International Conference "Agriculture for Life, Life for Agriculture" Section 4 Veterinary Medicine, Bucharest, Scientific Works. Series C, Veterinary Medicine, 2015, LXI, 2, 76-80.
8. **Goethe, R.,** Die demodikose des hundes eine Faktor krankheit?, Berliner Münchener Tierärztliche Wochenscrift, 1989, 102, 4, 293.
9. **Gortel, K.,** Update on canine demodicosis, Veterinary Clinics of North America: Small Animal Practice, 2006, 36, 1, 221-241.
10. **Mederle, N.,** Abordări terapeutice inovative în controlul unor parazitoze și implicațiile lor sociale, economice și asupra calității vieții, Teză de abilitare USAMVBT, 2018.
11. **Mederle, N., Mederle, O., Morariu, S., Herman, V., Negrescu, A., Marin, A.M., Suici, T., Dărăbuș, G.,** Study regarding the role and medical and social implications of *Demodex spp*. parazitism in dogs and humans, Revista Română Medicină Veterinară, 2020, 30, 2, 39-46.
12. **Mircean, V.,** Cercetări etio-morfologice în infecţiile cu dermatofiţi la carnivore, Teză de doctorat, Cluj-Napoca, 2003.
13. **Morariu, S., Dărăbuș, G., Oprescu, I., Mederle, N., Ilie, M.S., Imre, K., Imre, M.,** First evidence of the presence of *Demodex cornei* in Western Romania - Case report, Lucrări Științifice Medicnă Veterinară Timișoara, 2011, 44, 1, 38-41.
14. **Mueller, R.S., Meyer, D., Bensignor, E.,** Treatment of canine generalized demodicosis with a spot-on formulation containing 10% imidacloprid and 2.5% moxidectin (Advocate, Bayer Healthcare), Veterinary Dermatology, 2009, 20, 5-6, 441-446.
15. **Nayak, D.C., Tripathy, S.B., Dey, P.C., Ray, S.K., Mohanty, D.N., Parida, G.S., Biswal, S., Das, M.,** Prevalence of canine demodicosis in Orissa (India), Veterinary Parasitology, 1997, 73, 3/4, 347-352.
16. **Nwoha, R.I.O.,** Demodecosis in a dog, African Journal Clinical and Experimental Microbiology, 2011, 12, 3, 133-135.
17. **O'Neill, D.G., Turgoose, E., Church, D.B., Brodbelt, D.C., Hendricks, A.,** Juvenile-onset and adult-onset demodicosis in dogs in the UK: prevalence and breed associations, Journal Small Animal Practice, 2020, 61, 1, 32-41.
18. **Raczynski, E.,** Microscopic examination of skin scrapings in dogs, Zycie Weterynaryjne, 1996, 71, 9, 311-312.
19. **Radbea, N.,** Demodicoza Canina, Ed. Aura, Timișoara. 2005.
20. **Rahman, M., Bostami, M.B., Datta, A., Al Momen Sabuj, A., Rana, E.A., Mannan, A., Hossain, M.A., Elahi, Chowdhury, M.Y.,** Estimation of the prevalence and determination of risk factors associated with demodicosis in

dogs, Journal of Advanced Veterinary and Animal Research, 2021, 8, 1, 116-122.
21. **Shchelkanov, M.Y., Tabakaeva Moskvina, T.V., Kim, E.M., Derunov, D.A., Galkina, I.V.,** The prevalence and risk factors of canine demodicosis: A retrospective long-term study of 409 cases, Tropical Biomedicine, 2020, 37, 3, 778-782.
22. **Shrestha, D., Thapa, B., Rawal, G., Dhaka, S., Sharma, B.,** Prevalence of demodectic mange in canines of Kathmandu valley having skin disorder and its associated risk factors, International Journal of Applied Science Biotechnology, 2015, 3, 3, 459-463.
23. **Sivajothi, S., Sudhakara Reddy, B., Rayulu, V.C.,** Demodicosis caused by *Demodex canis* and *Demodex cornei* in dogs, Jorunal of Parasitogy Diseases, 2015, 39, 4, 673-6.
24. **Taylor, M., Coop, R.L., Wall, R.L.,** Veterinary Parasitology, 4th edition, Wiley-Blackwell, Oxford, 2015.
25. **Xhaxhiu, D., Kusi, I., Rapti, D., Visser, M., Knaus, M., Lindner, T., Rehbein, S.,** Ectoparasites of dogs and cats in Albania, Parasitology Research, 2009, 105, 6, 1577-87.

IDENTIFICATION OF *DEMODEX SPP.* PRESENCE IN DOG INFESTED WITH OTHER PARASITES. CASE REPORT

NEGRESCU A., MEDERLE N., MARIN A.M., DĂRĂBUŞ Gh.

Banat's University of Agricultural Sciences and Veterinary Medicine" King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645,
Calea Aradului No.119, Timisoara, Romania
E-mail: negrescu.adina-mh@ansvsa.ro

Summary

Demodicosis in one of the most prevalent diseases which affects the skin of dog. The etiological agent, *Demodex* mite could parasitize alone, or could be associated with different factors like poor condition, corticoterapy, imunitary system decrease, the presence of endo/ecto parasits. The study relates a case, dog, Cane Corso/Metis, 2.5 months old, male, kept in the yard and diagnosed with demodicosis. The presence of ticks, diptera larvae, fleas, roundworm eggs and protozoan oocysts was identified. One of the favorable factors involved in the mite multiplication and the clinical manifestation of the disease could be spoliation of the body through endoparasitism and the presence of different ectoparasits.
Keywords: *Demodex spp.,* dog, associated parasitism.

Demodex canis, the most well-known and studied species of the genus, affects the hair follicle and the sebaceous gland and causes the appearance of one of the most important skin diseases in dogs (16).

When the mite is associated with the bacterial flora, the dry lesions become moist, and the disease assumes the character of pyodemodicosis. There are also situations in which *Demodex canis* evolves, on the same individual, associated with other etiological agents of a parasitic nature (fleas, ticks, cestodes, nematodes). These situations must be identified quickly because the animal's health is affected, and the host's fight against an accumulation of parasitic agents can end, in the most frequent cases, with a failure for the parasitized animal (2).

The case study describes the evolution of demodicosis in a dog in which other parasitic etiological agents were also identified.

Materials and methods

Case presentation - dog, Cane Corso/Metis, 2.5 months old, male, kept in the yard.

Clinical, dermatological and laboratory examinations were performed: skin scraping, scotch tape examination, trichogram, coproparasitological examination (flotation method), examination under a magnifying glass and under a microscope of the samples taken (Fig. 1).

Fig. 1. Clinical examination

Results and discussions

The results of the clinical examination revealed the presence of dry demodicosis lesions. The presence of ticks, diptera larvae and fleas was identified (Fig. 2, Fig. 3).

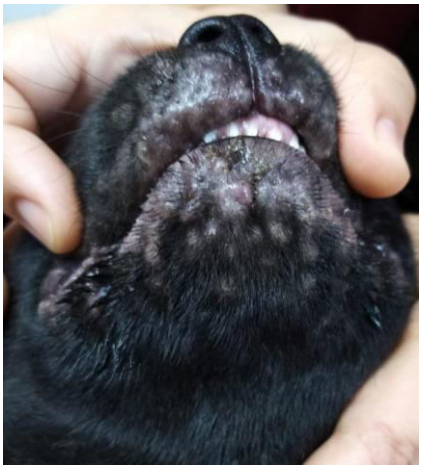


Fig. 2. Demodicosis lesions

Fig. 3. Diptera larvae

The coproparasitological examination highlighted the presence of roundworm eggs and protozoan oocysts (Fig. 4, Fig. 5).

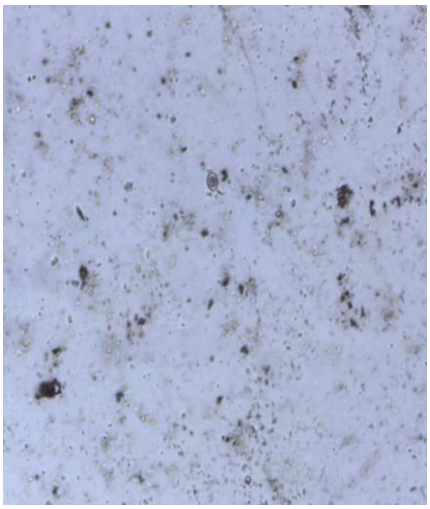


Fig. 4. Protozoan oocysts

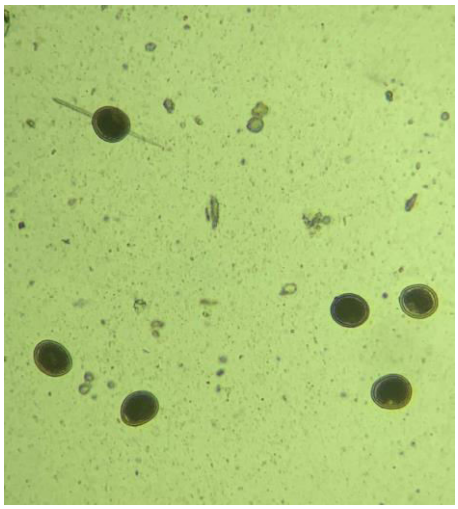


Fig. 5. Roundworm eggs

The microscopic examination of the skin scrapings made from the lesions confirmed the presence of the *Demodex* mite (Fig. 6).

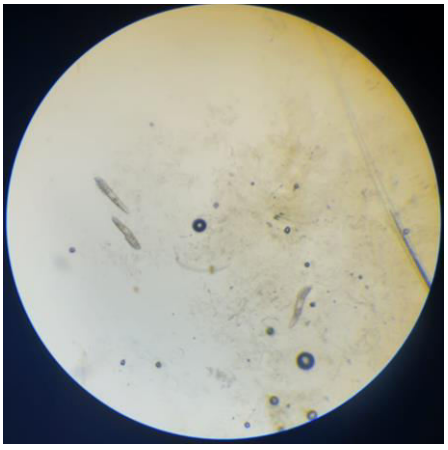


Fig. 6. *Demodex spp.*

The results of the present study are in agreement with the results obtained by other authors who support the important role that the etiology associated with the mite obviously influences the outbreak and evolution of demodicosis. A multifactorial disease, as demodicosis is described, involves in its initiation and progression a series of intrinsic and extrinsic factors (the state of the host's immune system, the presence of endoparasites, prolonged corticosteroid treatments, maintenance conditions, age) (2, 5, 8, 13, 14).

Some authors (6, 7, 9, 10) indicate that the associated evolution between *D. canis* and different species of dermatophytes is possible: the fungi adhere to the corneocytes, then invade the hair shaft, producing disturbances in the epidermal homeostasis , a favorable aspect for the *Demodex* mite, which "takes advantage" of the opportunity to colonize the skin.

The evaluation of the factors involved in the initiation of canine demodicosis highlighted the importance of endoparasitism with various species (*Leishmania infantum, Dipilidium caninum, Echinococcus graulosus, Toxocara canis, Ancylostoma braziliense, Trichuris vulpis, Dirofilaria immitis*), of associated parasitism (*Ctenocephalides canis, Otodectes cynotis, Sarcoptes scabiei, Rhipicephalus sanguineus, Tunga penetrans*), as well as the biodiversity of the environment in which the parasitized animal lives (3, 4).

The statistical analysis applied to a batch of 409 dogs reveals a positive correlation between the occurrence of demodicosis, age and associated parasitism. Dogs over three years of age, parasitized with different species of cestodes are the most prone to demodectic infestation (15).

If on the American continent, the monitoring of demodicosis over a period of 16 years led to the highlighting of the association of acariasis with allergic dermatitis (1), but also with the presence of coccidiosis and different species of cestodes (12), on the Australian continent, the associated pathogenesis is represented by hypothyroidism and leishmaniasis (11).

The present case study reveals a direct correlation between the dog's age (2.5 months) and the clinical manifestation of localized demodicosis (DL) against the background of the decrease in the host's defense reaction due to the presence of protozoa and roundworms. This picture is completed by the damage to the skin's immune system through infestation with ectoparasites (fleas, ticks, diptera larvae), a situation also due to the dog's maintenance conditions (in the yard, the countryside).

Conclusions

Young age, poor maintenance conditions, spoliation of the body through endoparasitism and the presence of ticks, fleas and myazigenous larvae that do not compete with *Demodex* for space or food are favorable factors in the multiplication of the mite and the clinical manifestation of the disease.

Acknowledgments

This scientific paper was carried out in the Laboratory of Parasitology and Parasitic Disease at the Faculty of Veterinary Medicine Timisoara, Laboratory within the Animal Hygiene and Pathology Research Center/ BUASVM Timisoara.

References

1. **Bowden, D.G., Outerbridge, C.A., Kissel, M.B., Baron, J.N., White, S.D.,** Canine demodicosis: a retrospective study of a veterinary hospital population in California, USA (2000-2016), Veterinary Dermatology, 2018, 29, 1, 19-e10.
2. **Chen, C.,** A short tailed demodectic mite and *Demodex canis* infestation in a Chihuahua dog, Veterinary Dermatology, 1995, 6, 4, 227-229.
3. **Dantas-Torres, F., Otranto, D.,** Dogs, cats, parasites, and humans in Brazil: opening the black box, Parasites&Vectors, 2014.
4. **Dărăbuş, G., Oprescu, I., Morariu, S., Mederle, N.,** Parazitologie și boli parazitare. Ed. Mirton, Timișoara, 2006.
5. **Kumari, P., Nigam, R., Choudhury, S., Singh, S.K., Yadav, B., Kumar, D., Garg, S.K.,** *Demodex canis* targets TLRs to evade host immunity and induce canine demodicosis, Parasite Immunology, 2018, 40, 3.
6. **Lockwood, S.L., Mount, R., Lewis, T.P., Elliot, Schick, A.,** Concurrent development of generalized demodicosis, dermatophytosis and meticillin-resistant *Staphylococcus pseudintermedius* secondary to inappropriate treatment of atopic dermatitis in an adult dog, Companion or pet animals, 2017, 5, 1.
7. **Mason, I.S., Mason, K.V., Lloyd, D.H.,** A reviewe of the biology of canine skine with repect to the commensals *Staphylococcus intermedius. Demodex canis* and *Malassezia pachydermatis*, Veterinary Dermatology 1996, 7, 1, 119-132.
8. **Mederle, N., Dărăbuș, G., Oprescu, I., Morariu, S., Ilie, M., Indre, D., Mederle, O.,** Diagnosis of canine demodicosis, Science Parasitology, 2010, 11, 1, 20-23.
9. **Moriello, K.A., Coyner, K., Paterson, S., Mignon, B.,** Diagnosis and treatment of dermatophytosis in dogs and cats: Clinical Consensus Guidelines of the World Association for Veterinary Dermatology, Veterinary Dermatology, 2017, 28, 266-e268.
10. **Morris, D.O., Loeffler, A., Davis, M.F., Guardabassi, L., Weese, J.S.,** Recommendations for approaches to meticillin-resistant staphylococcal infections of small animals: diagnosis, therapeutic considerations and preventative measures: Clinical Consensus Guidelines of the World Association for Veterinary Dermatology, Veterinary Dermatology, 2017, 28, 304-e369.

11. **Pinsenschaum, L., Chan, D.H.L., Vogelnest, L., Weber, K., Mueller, R.S.,** Is there a correlation between canine adult-onset demodicosis and other diseases?, Veterinary Record, 2019, 185, 23, 729.
12. **Plant, J.D., Lund, E.M., Yang, M.,** A case-control study of the risk factors for canine juvenile-onset generalized demodicosis in the USA, Veterinary Dermatology, 2011, 22, 1, 95-9.
13. **Radbea, N.,** Demodicoza Canina, Ed. Aura, Timișoara. 2005.
14. **Rahman, M., Bostami, M.B., Datta, A., Al Momen Sabuj, A., Rana, E.A., Mannan, A., Hossain, M.A., Elahi, Chowdhury, M.Y.,** Estimation of the prevalence and determination of risk factors associated with demodicosis in dogs, Journal of Advanced Veterinary and Animal Research, 2021, 8, 1, 116-122.
15. **Shchelkanov, M.Y., Tabakaeva Moskvina, T.V., Kim, E.M., Derunov, D.A., Galkina, I.V.,** The prevalence and risk factors of canine demodicosis: A retrospective long-term study of 409 cases, Tropical Biomedicine, 2020, 37, 3, 778-782.
16. **Tamura, Y., Kawamura, Y., Inoue, I., Ishino, S.,** Scanning electron microscopy description of a new species of *Demodex canis spp.*, Veterinary Dermatology, 2001, 12, 5, 275-8.

AUTHORS INDEX

CONTENT