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THE EFFECT OF SOME PHYSICAL-CHEMICAL FACTORS ON *YERSINIA ENTEROCOLITICA* STRAINS

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Summary

In recent years, obtaining healthy food has become extremely important, therefore new rules and standards for food hygiene control have been introduced, as processed foods can be an important risk factor by being involved in a number of pathological conditions. The aim of this research was to observe the tolerance level for some strains of *Yersinia enterocolitica*, isolated from pigs feces, to certain temperature variations as well as to the action of sodium chloride. The experiments were performed on five strains of *Yersinia enterocolitica*, isolated from pigs, after processing 30 samples, collected directly from the rectum. The obtained results confirm the sensitivity of the bacterium to low temperatures as well as to hyperchlorinated media.

Keywords: salmonella, chicken, incidence

Yersinia enterocolitica is a bacteria included in the *Enterobacteriaceae* family, widespread in the environment, which has been frequently isolated from the intestinal tract of many species of domestic and wild mammals, but also from rodents, birds, fish, frogs, mollusks, crustaceans and humans.

Recently, more and more researchers include *Yersinia enterocolitica* in the foodborne pathogens group, which can contaminate most food products, including pork, beef and lamb, milk and dairy products, especially raw milk, pasteurized milk, powdered milk, cream and ice cream, vegetables, seafood etc. (2, 6, 11).

Based on these facts, it is considered that an important role in preventing the contamination of food products from animals belongs to the ways of preserving them in order to avoid contamination. Given these aspects, although freezing is considered extremely important, literature data suggest that low temperatures play an important role in slowing down meat spoilage processes. This statement is supported by the fact that most food pathogens do not multiply in frozen products and gradually lose their viability although their enzyme systems are relatively resistant and remain active even at a negative temperature of -30°C (3, 4, 5, 7, 8, 10).

Materials and methods

A number of five bacterial strains of the *Enterobacteriaceae* family and included in *Yersinia enterocolitica* species, isolated and identified from 30 pigs samples, were studied for research. The samples were collected from the rectum of pigs, using sterile cotton swabs. Next, for the isolation and identification of bacterial strains, all 30 samples were initially inoculated on common culture media (meat broth and nutrient agar) and enrichment media (PSB and ITC), and subsequently on selective media for *Enterobacteriaceae*.

After performing the laboratory tests, the strains that were identified and included in *Yersinia enterocolitica* species were also subjected to biochemical tests, after which they were reseeded in simple broth to study the behavior of physical-chemical factors, respectively freezing - refrigeration, according to the working protocol shown in Table 1, as well as the action of NaCl.

We mention that the strains sown in meat broth were exposed to temperatures between -18°C and $+4^{\circ}\text{C}$, for a period between two and 30 days, according to the working protocol presented in Table 1.

To determine the resistance to chemical factors, the effect of NaCl on bacterial populations of *Yersinia enterocolitica* in a liquid media (meat broth) was studied, to which different concentrations of NaCl were used.

Table 1

Working protocol

No. of strains	CFU/ml media	Specification		
		Temperature	Period	No. of samples
5	10^6	-18°C	20 days	5
			30 days	5
		-12°C	20 days	5
			30 days	5
		-4°C	20 days	5
			30 days	5
		0°C	2 days	5
			4 days	5
		$+4^{\circ}\text{C}$	2 days	5
			4 days	5

Results and discussions

The obtained results showed that at a temperature of 4°C the number of viable bacterial cells is constant, but, as the freezing temperature decreases, there is a significant decrease in the number of viable bacterial cells (from 10^6 to 10^2). It was also found that, at refrigeration temperature, the strains remain viable, but with

a significant numerical decrease to 0°C compared to 4°C, a temperature at which even a slight multiplication process is observed (Fig. 1).

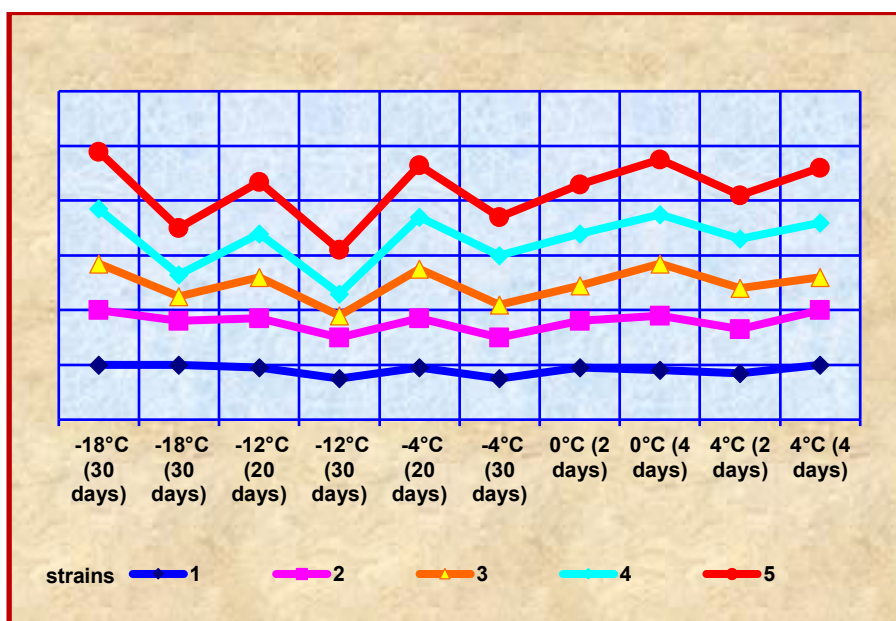


Fig. 1. The multiplication dynamics of *Y. enterocolitica* strains at freezing and refrigeration temperature

We also mention the important role of the period of exposure to low temperatures. For example, between the samples maintained for 20 days at -18°C and those maintained for 30 days at the same temperature, the differences in viability varied between one and three logarithms. Analyzing the data obtained we can say that if the exposure period was shorter (for example, two and four days), the differences in viability of *Yersinia enterocolitica* strains were almost insignificant. Moreover, in the case of the samples subjected to refrigeration for a longer period of time, a logarithmic increase of the strains was found (Fig. 1).

In conclusion, we can mention that with the increase of the exposure time at freezing temperatures (-18°C) the number of viable bacterial cells decreases, while at refrigeration temperatures there was an increase in their number, which was different from a strain to another. The most constant values were recorded in the case of strains 1 and 2, and the obtained results are similar to those published in the literature (1, 4, 9).

Regarding the resistance to the action of NaCl, the obtained results showed that *Yersinia enterocolitica* strains tolerate NaCl concentrations of up to 5%. Above

this percentage, regardless of the concentration used, the death of the culture occurs. For example, at a concentration of 7% NaCl, after 72 hours of exposing the culture in the hyperchlorinated medium, the recovery of the strains by reseeded in nutrient broth failed. We mention that the evolution of the studied strains was different depending on the salt concentration of the culture media (Fig. 2).

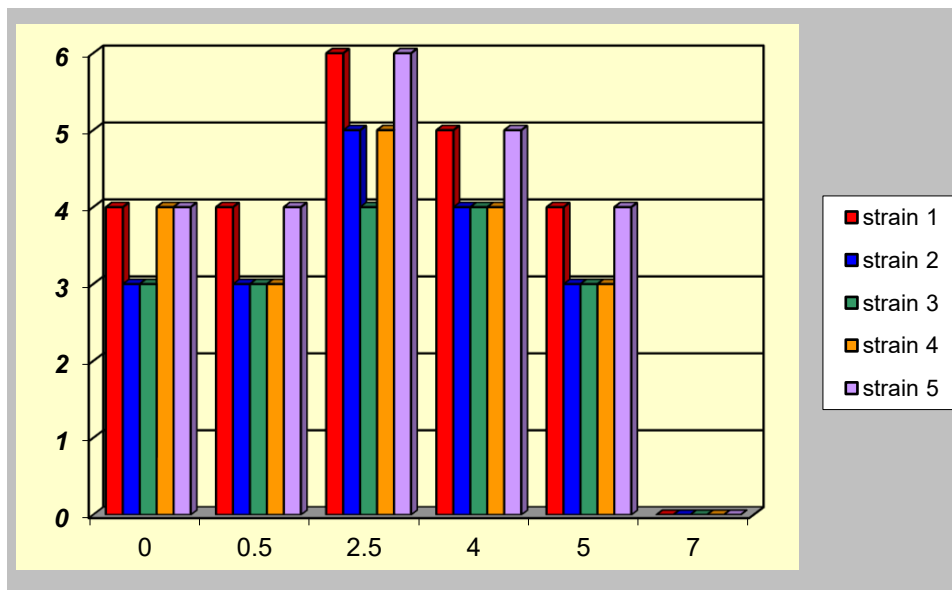


Fig. 2. The multiplication dynamics of *Yersinia enterocolitica* strains under the action of salt

The maximum recorded number of viable cells, compared to the initial values, was found at a salt concentration of the culture medium between 2.5% and 4%. This confirms that *Yersinia enterocolitica* strains tolerate relatively high salt concentrations. As can be seen, the increase in salt concentration in the culture medium is inversely proportional to the germination concentration (CFU/ml), reaching zero CFU/ml at a concentration of 7% NaCl and, as mentioned above, after a 72 hours incubation at 37°C.

Conclusions

Yersinia enterocolitica is a bacteria sensitive to freezing, therefore, at a temperature of -18°C, the number of bacterial cells in pork is reduced by at least one logarithm after 20 and 30 days.

At refrigeration temperatures (0°C and 2 – 4°C), *Yersinia enterocolitica* multiplied, the multiplication being observed on raw pork, a product in which an increase in the number of cells by a maximum of one logarithm, was observed.

Based on the obtained results, we can say that in case of massive contamination with *Yersinia enterocolitica*, the storage time is not always enough to ensure healthy products for consumers.

In order to enhance the bactericidal action of the freezing process of the products, we consider necessary their ventilation, in order to create a dry surface at the surface of the products.

The concentration of 4% NaCl proved to be a concentration which limited the bacterial development, at 5% NaCl, the survival of the germs was still possible, and at the concentration of 7% NaCl a total bactericidal effect was observed on all the studied strains.

NaCl reduces the bacterial population when its concentration is above 5%. This aspect is worthy of consideration in practice, being of real use in the sausage making sectors in slaughterhouses, where the processing of these products, in terms of hygiene, raises major problems.

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A SURVEY ON ANAPLASMOSIS IN DOGS FROM THREE COUNTIES OF ROMANIA

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Summary

Canine anaplasmosis is a vector-borne disease caused by *Anaplasma phagocytophilum*, transmitted by Ixodid ticks and maintained in a variety of mammal species. The present study investigated the seroprevalence of *Anaplasma phagocytophilum* in dogs from three counties of Romania. Between 2015 and 2019, blood samples were collected from 92 dogs originating in Sibiu (n=17), Tulcea (n=26) and Iași (n=49) counties. All the serum samples were tested using a commercial enzyme-linked immunosorbent assays (ELISA) with the purpose to detect anti-*Anaplasma phagocytophilum* IgG antibodies. The results of the investigations revealed an overall prevalence of 14.13% (13 out of 92 tested dogs were identified as seropositive). The highest seroprevalence was recorded in hunting dogs from Agnita-Sibiu (10 positive dogs out 13 tested), suggesting a higher risk of exposure to ticks. Our results highlight the presence of canine anaplasmosis in the studied area and the importance of use of tick preventive product on dogs. In conclusion, the findings of this study indicate that veterinarians should be aware of the existence of *Anaplasma phagocytophilum* and consider her in the differential diagnosis of dogs showing vector-borne diseases clinical signs or laboratory abnormalities. Alongside the serological investigation, diagnosis of canine anaplasmosis should be always based on clinical signs and laboratory investigations.

Keywords: Canine anaplasmosis, dogs, ELISA, IgG

Anaplasma phagocytophilum (*A. phagocytophilum*) previously named *Ehrlichia phagocytophila* or *Ehrlichia equi* is an emerging pathogen of humans, horses and dogs worldwide with the seroprevalence varies from region to region. *A. phagocytophilum* is a Gram-negative, obligate intracytoplasmic coccus that belongs to the family of the *Anaplasmataceae* under the order *Rickettsiales*. It is found in cytoplasmic vacuoles forming distinctive inclusion bodies, called morulae. *A. phagocytophilum* mostly attacks neutrophilic and rarely eosinophilic granulocytes (1) and it is transmitted by ticks of the genus *Ixodes*: *Ixodes ricinus* in Europe (2) and *Ixodes scapularis* and *Ixodes pacificus* in the United States (3). Rarely, transmission of the organism has been reported in the absence of a tick vector, but nosocomial transmission was reported in China (4).

The bacteria were first described as a veterinary pathogen, after its identification in the cytoplasm of leukocytes of sheep in Scotland (5, 6). *A. phagocytophilum* is the causative agent of diseases such as canine, feline, equine, and human granulocytic anaplasmosis and of tick-borne fever in ruminants (7). The

early cases in dogs were described in the United States in 1982 and in Europe in 1988 (8).

The pathogenesis of granulocytic anaplasmosis is not fully understood. Transmission of *A. phagocytophilum* from the tick to animals is accomplished in approximately 24-48 hours after the tick bite (9) and it spreads within the host through blood or lymph (10). The arthropod seems to release anticoagulants, anti-inflammatory and potent salivary immune-modulators facilitating the establishment of the transmitted pathogen (11). The most common clinical signs in dogs that develop illness are lethargy and fever. Lethargy has been reported in almost all dogs affected (12, 13). Musculoskeletal signs, such as reluctance to move and lameness, also have been commonly reported. Antibodies against *A. phagocytophilum* occur in the serum of infected animals after 7 to 14 days post-exposure. In experimentally infected dogs, morulae appear as early as 4 days after inoculation, and persist for 4-8 days (14).

Contrary to common beliefs, many dogs which show specific antibodies against *A. phagocytophilum* are not clinically conspicuous. In dogs, the diagnosis is based on serological detection of antibodies using ELISA, indirect immunofluorescence or rapid test. The detection of morulae inside the granulocytes by Giemsa or Wright stain is also possible, but only a small percentage of the cells are infected and shortly after the beginning of treatment the morulae cannot be determined. Culture is complicated and could not yet be performed reproducibly. Detection by PCR is regularly used but the sensitivity of the direct detection depends on the phase of infection. However, the detection of antibodies is therefore still a method of choice for laboratory diagnostics of canine granulocytic anaplasmosis.

In Romania, there are still not sufficient data concerning canine anaplasmosis. Serological surveys conducted in dogs for the detection of specific antibodies against *A. phagocytophilum* offer a promising perspective in identifying the potential risk areas for humans. The aim of this study was to investigate the seroprevalence of *A. phagocytophilum* IgG antibodies in dogs from three counties in Romania, between 2015-2019.

Materials and methods

In this study, the population consisted of 92 dogs of which 17 were hunting dogs from Agnita (Sibiu County), 26 dogs kept in shelters in Tulcea County and 43 dogs from Iași County (32 samples from Tomesti shelter and 17 samples from dogs examined in veterinary private clinics) (Table 1).

Blood samples were collected aseptically from the cephalic vein in tubes with clot activator. The serum samples were centrifuged for 10 minutes at 3000 rotations per minute (RPM) and stored at – 20°C until testing. In all cases, informed consent was obtained from the owners and from the shelter veterinarian.

Dog serums were analyzed using commercial enzyme-linked immunosorbent assays with the purpose to detect specific antibodies of the

immunoglobulin G class (IgG) *Anaplasma phagocytophilum* (Anti-*Anaplasma phagocytophilum* ELISA Dog IgG, Euroimmun 23560 Lübeck, Germany).

Table 1

Distribution of the tested dogs by county

County	Number of samples		
	Males	Females	Total
Sibiu	12	5	17
Tulcea	20	6	26
Iași	30	19	49
Total	62	30	92

Microtiter strip wells are coated with recombinant antigen of *Anaplasma phagocytophilum* to bind corresponding antibodies of the specimen. Serum samples were diluted 1:101 according to the manufacturer's instructions. Absorbance values were measured using a Microplate Reader (Tecan Sunrise, Tecan Trading AG, Switzerland) at 450 nm with 620 nm as a reference wave length. According to the manufacturer, the test has a sensitivity of 97% and a specificity of 96%.

Results and discussions

Canine anaplasmosis is a vector-borne disease which has a major significance for canine health. In addition to its veterinary importance, the disease has a zoonotic potential, posing a risk of transmission to humans (15). The objective of the study was to evaluate the burden of infection with *Anaplasma phagocytophilum* in the different dog populations from three counties of Romania. Dogs age spectrum varied from 3 months to 13 years. Details regarding age, origin and living conditions were also collected. Following the ELISA testing, 13 out of 92 (14.13%) samples were identified as positives for specific *A. phagocytophilum* IgG antibodies.

Differences in seroprevalence between locations were registered. In hunting dogs from Agnita (Sibiu county) was revealed the highest prevalence, 10 out of 17 (58.8%) tested samples being *A. phagocytophilum* IgG. In case of the dogs from Tulcea county, 2 out of 26 (7.7%) samples were seropositive. In Iasi county, the *A. phagocytophilum* IgG prevalence registered in this study was the lowest, 1 out 49 (2%) samples being identified as seropositive. Correlating the obtained results with gender, 8 out of 62 males (12.9%) and 5 out of 30 females (16.7%) were *A. phagocytophilum* IgG positive. Two dogs from Iași county and one from Sibiu county were identified with a borderline result.

Table 2

Signalment and origin of the *A. phagocytophilum* IgG positive dogs

Gender and age	County	<i>A. phagocytophilum</i> IgG antibodies
Female, 13 years	Agnita-Sibiu	+
Female, 7 years	Agnita-Sibiu	+
Female, 2 years	Agnita-Sibiu	+
Male, 2 years	Agnita-Sibiu	+
Male, 3 years	Agnita-Sibiu	+
Female, 9 years	Agnita-Sibiu	+
Male, 4 years	Agnita-Sibiu	+
Male, 10 years	Agnita-Sibiu	+
Female, 3 years	Agnita-Sibiu	+
Male, 8 years	Agnita-Sibiu	+
Male, 7 years	Agnita-Sibiu	±
Male, 1 year	Tomesti-Iasi	+
Male, 3 years	Tomesti-Iasi	±
Female, 2 years	Tomesti-Iasi	±
Male, 7 years	Tulcea	+
Male, 2 years	Tulcea	+

*Legend: +: positive sample; ±: borderline

During recent decades, the worldwide distribution of canine vector-borne diseases has been constantly changing (16). This changes are attributed to climate factors, globalization, international transportation and trade, canine and wildlife reservoir populations (17). The most important factor considering the spread of vectors and the distribution of vector-borne diseases is the climate because the arthropod vectors are strongly affected by environmental temperature (18).

The present study demonstrated the presence of canine anaplasmosis in three counties from Romania, between 2015-2019. Data regarding granulocytic anaplasmosis in dogs in Romania are limited. In 2012, Mircean et al. (19) tested 1146 samples from 16 counties, collected from randomly selected dogs, using ELISA SNAP 4DX (IDEXX Laboratories, Inc., Westbrook, ME). This study revealed a lower seroprevalence for *A. phagocytophilum* (5.5%), compared to our findings. Also, a recent study performed by our team in 2019, revealed a value of 5.43% for *A. phagocytophilum* IgG antibodies (20). These differences can be explained by the fact that in the recent work were included hunting dogs living outdoors who received only occasionally antiparasitic treatments, this fact increasing the chance of being exposed to vectors. High seroprevalence in the canine population suggests that subclinical *A. phagocytophilum* infection or mild disease due to infection is common in regions where ixodid tick density is high. Many dogs infected with vector-borne agents remain asymptomatic for months or even years, but diagnosis of subclinical infection is important, as those animals might still serve as reservoirs of pathogens to other hosts including humans.

Several studies have reported the presence of *A. phagocytophilum* in Europe, by using various diagnostic methods (15, 21, 22). A study carried out in Bulgaria, in 2015 (22) showed a much higher seroprevalence (46.1%). Another study from Germany, published a higher prevalence (43%) after testing 522 dogs (21). These findings could be related to the climatic conditions, which are known to strongly influence the presence of ticks, the increasing vector exposure depending on animals' age and/or immunological status (23). Furthermore, the seroprevalence values recorded in dogs in Italy, from 2013 to 2017, was lower for *A. phagocytophilum* (3.31%) compared to our results (24). A lower prevalence (2.7%) was reported also in Greece, in 2019 (15).

Conclusions

The prevalence of IgG antibodies anti *A. phagocytophilum* obtained in this study was higher compared to other studies conducted in Romania. Anaplasmosis is highly prevalent in Sibiu County and should be a disease that any clinician should add to his differentials in dogs with unspecific illness. The fact that we included in our research hunting dogs, which come close to specific habitats of ticks, can be the explanation for our findings.

Our results suggest that dogs living outdoors and receiving no treatments are prone to infection with *A. phagocytophilum* and highlight the importance for appropriate antiparasitic treatments. Veterinarians should consider this pathogen in the case of suggestive clinical signs and in routine health status checks.

Collaborative research efforts by those in public health and veterinary medicine could facilitate understanding of canine anaplasmosis and other tick-borne pathogens that cause disease in both dogs and humans. To conclude, because there is little serologic evidence of tick-transmitted diseases in Romania, more serological surveys need to be performed in order to establish the potential risk of canine anaplasmosis for dog population.

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**EPIDEMIOLOGIC EVALUATION OF CANINE AND FELINE
UROLITHIASIS IN A VETERINARY CLINIC FROM TIMISOARA,
ROMANIA, DURING 2016 - 2019**

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Summary

Urolithiasis is a common and recurrent disease in dogs and cats, manifested through dysuria, urinary tract infections, partial or total urinary obstruction and polyp formation. The aim of this paper is to present local epidemiological data on urolithiasis in dogs and cats, and to assess the risk factors that may lead to disease occurrence. Thus, 114 patients (46 dogs and 68 cats) with urolithiasis, from a veterinary clinic in Timisoara, were registered. The patients belonged to various breed and age groups, either displaying various diseases or being presented for the first time in the clinic. The assessment of risk factors did not reveal a statistically significant result regarding the influence of breed, sex or age on the occurrence of urolithiasis ($p > 0.05$). For the recorded patients, the urolithiasis recurrence rate was higher than data mentioned in specialty literature. There was no evidence suggesting a statistically significant relationship between antibiotic therapy and the presence of urolithiasis.

Keywords: urolithiasis, dog, cat, epidemiology, antibiotic therapy

Urolithiasis is a common and recurrent disease in dogs and cats, with an estimated prevalence of 0.4% – 2.0% in dogs (13) and approximately 7% in cats. Urolithiasis has a high variability, depending on breed, sex, age, or geographical area (19).

Urolithiasis is a condition characterized by stones found at any level of the urinary tract (15, 19, 22). Urolith formation is favored by urinary pH, low water intake in relation to the degree of urine concentration, vitamin A deficiency and urinary infection (14, 18, 20). The size and shape of the stones are variable depending on the species, the place of formation or composition (18).

Not all urinary stones show clinical signs, but when they do occur, they can be dysuria, urinary tract infections, partial or total urinary obstruction, and polyps (2, 7). The symptoms noticed in case of kidney stones are hematuria and pyelonephritis or pyelonephrosis specific symptoms such as: anorexia, lethargy, fever, polyuria/polydipsia or pain (3, 5, 8, 15, 20).

Urinary stones can lead to urinary tract infections due to traumatic damage to the bladder lining, incomplete emptying of the bladder or sequestration of microorganisms. Urinary tract infections are often associated with urinary stones, and are a determining factor for struvite formation in dogs. Once formed, struvite stones can help maintain and spread the infection in the urinary tract (2, 7).

Small stones can block the urethra during the process of urine elimination, producing urinary obstruction (2, 3, 7, 8). Blockages in the urethra are common in males, the most common locations being the proximal end of the penile bone in dogs and anywhere along the cat's urethra (15). Uremia can also be a consequence of obstruction. When associated with urinary tract infection, urolithiasis can lead to the destruction of the renal parenchyma and sepsis, followed by death in 2 – 4 days if left untreated (2, 7).

The aim of this paper is to present local epidemiological data on urolithiasis in dogs and cats and to assess the risk factors that might lead to disease. In Romania, there is no database to support veterinarians with useful information in order to improve the quality of their medical act.

Materials and methods

The study was conducted in a private veterinary clinic in Timisoara, during 2016–2019, on 114 patients (dogs and cats) with urolithiasis. The patients were of various breed, age and sex; they were diagnosed with various diseases or presented in the clinic for the first time.

Urolithiasis was diagnosed by corroborating the clinical signs with the results obtained in paraclinical examinations (imaging examination - ultrasound, X-ray and microscopic examination of urine).

Clinical examination of the patients revealed one or more of the following urolithiasis associated symptoms: dysuria, stranguria, pollakiuria, anuria, hematuria, partial or total urinary obstruction, with the presence of bladder distension.

Ultrasound was performed in order to confirm the diagnostic, a technique which revealed signs such as thickening of the bladder mucosal lining and hyperechoic sediment (Fig. 1b) or stones (Fig. 1a). In case the ultrasound was not probative, the radiographic examination was used to identify the size and location of the urolith.

Microscopic examination of the urinary sediment was performed manually, through the usual method (22), without staining.

The Microsoft Excel program and the SPSS18 software were used for statistical analysis and data interpretation. The Chi-Square test was used to determine the significant differences in case of quantitative variables.

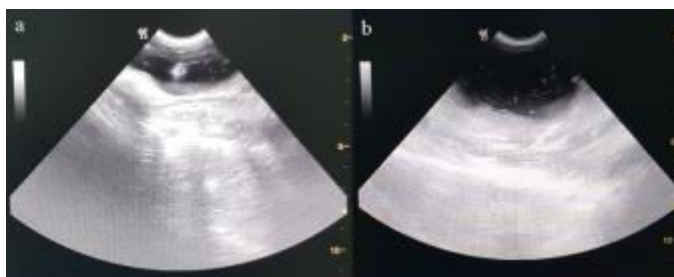


Fig. 1. Bladder urolithiasis (a) and sediment (b), dog

Results and discussion

Data revealed that 46 (40%) out of 114 patients were dogs and 68 (60%) were cats. In dogs, the distribution was equal for males and females (50%), whereas in cats, 85% of urolithiasis patients were males.

The probability for bitches to eliminate stones spontaneously, without clinical evidence, is quite high due to the anatomical features of the urethra in females (shorter and straighter path, larger diameter).

In dogs, the most affected breeds were Shi-Tzu (9 dogs), mixed-breed (7 dogs) and Pug (6 dogs) (Fig. 2).

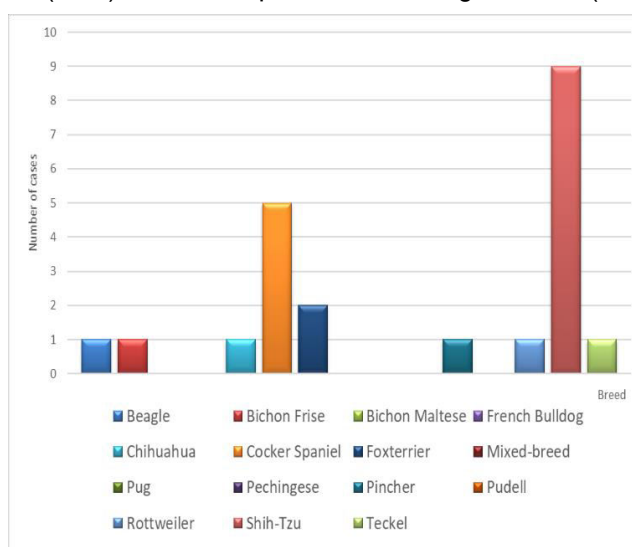


Fig. 2. Prevalence of urolithiasis in dogs, according to breed

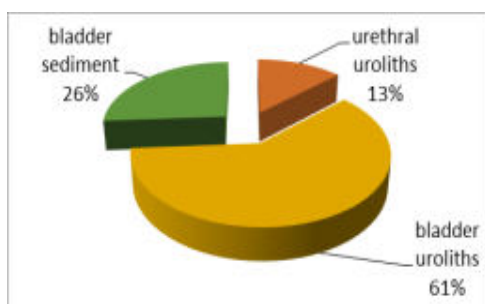


Fig. 3. Distribution of urolithiasis in dogs, according to diagnosis

patients (88%). A higher prevalence was followed by bladder stones (34%) (Fig. 6).

In dogs, 61% of the diagnosed uroliths were bladder stones, followed by bladder sediment (26%). Kidney stones were not identified at all, and urethral stones were less common as well (13%) (Fig. 3). Bladder stones were identified mainly in Shi-Tzu dogs (25%) and mixed-breed dogs (25%) (Fig. 4). Urinary sediment was diagnosed mainly in the Pugs (37%) and Bichon Maltese patients (18%) (Fig. 5).

In cats, urolithiasis was mainly identified in mixed-breed signaled for bladder sediment (49%),

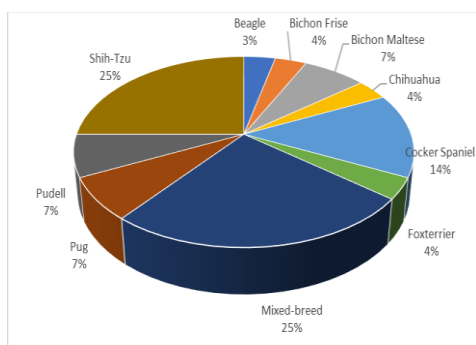


Fig. 4. Distribution of bladder stones in dogs, according to breed

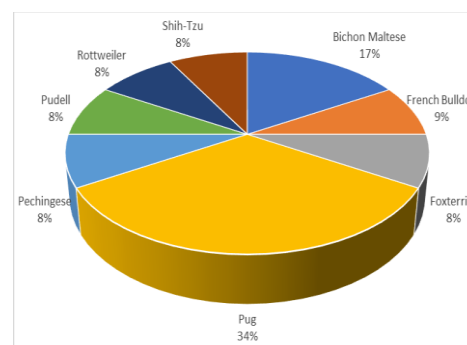


Fig. 5. Distribution of urinary sediment in dogs, according to breed

In terms of age category, in dogs, urolithiasis was predominantly diagnosed in 6–8 year old dogs, respectively, in 11 year old dogs. The lowest percentage was observed in young dogs (6 months) and in those over 11 years of age. In cats, the occurrence of urolithiasis was higher between 2 and 4 years of age, with a low rate in geriatric patients (Fig. 7). Unfortunately, in 5 dogs and 18 cats, the age was not recorded in the clinic's database due to technical errors.

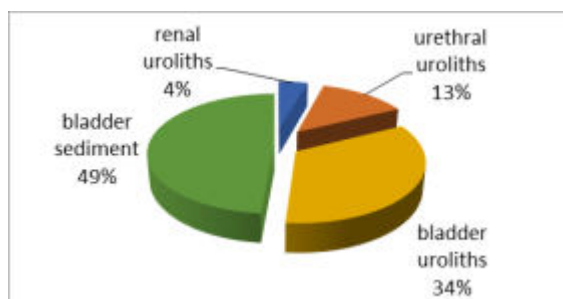


Fig. 6. Distribution of urolithiasis in cats, according to diagnosis

Bladder stones were identified in dogs aged 7 (21%) and 8 years (17%), while in the case of bladder sediment, the incidence was higher between 3 and 6 years. Thus, the evolution is ascending, from sediment to calculus, proportionally with aging. The same trend is observed in cats, where urinary sediment was mainly highlighted in younger individuals (2 years), and

stones in older ones (3 years).

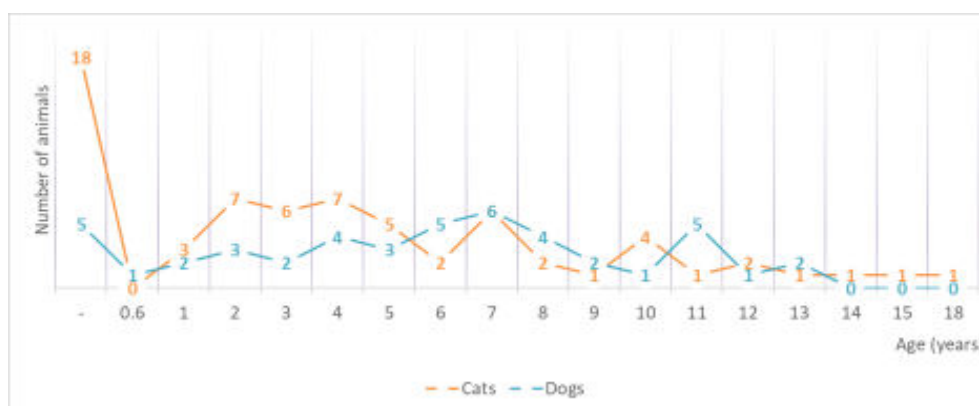


Fig. 7. Urolithiasis prevalence in cats and dogs, according to age; „-“ no data available

Urine samples were taken from 37 (28 cats and 9 dogs) of the 114 patients and struvite was identified as the only type of urolith. Struvite was mostly identified in males (83.78%) and less in females (16.22%).

The probabilities obtained by running the independence test were $p > 0.05$. Thus, urolithiasis was not significantly influenced by sex, breed or age.

In terms of disease history, 33% of the dogs had at least one recurrent episode over time (Fig. 8). In cats, the urolithiasis recurrence rate was 19% (Fig. 9). The majority of relapses in cats were seen in males (85%) and only 15% females.

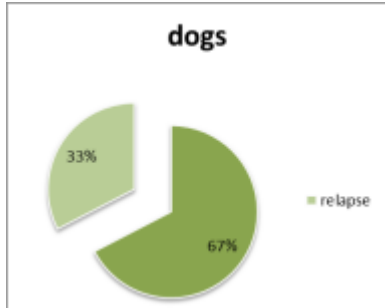


Fig. 8. Recurrence rate of urolithiasis in dogs

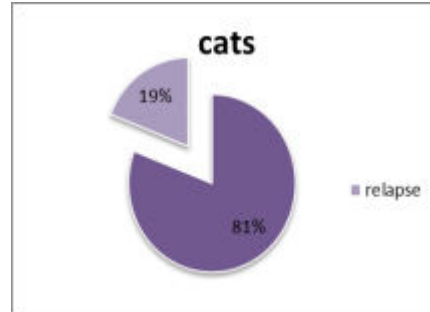


Fig. 9. Recurrence rate of urolithiasis in cats

Fifty patients out of 114 had a history of antibiotic administration (Synulox - 41, Enroxyl - 6, Stomorgyl - 3) (Fig. 10, 11).

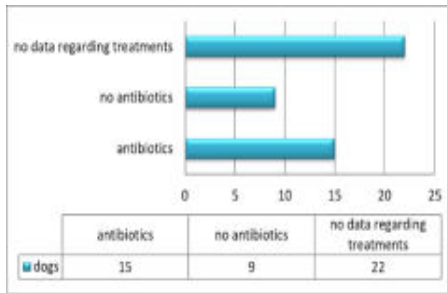


Fig. 10. Distribution of dogs according to antibiotic therapy

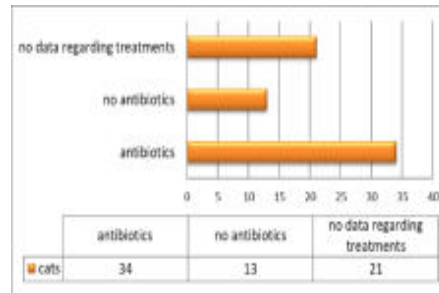


Fig. 11. Distribution of cats according to antibiotic therapy

They were divided into groups based on the following elements: type of urolithiasis (stones, sediment) and therapeutic history thus, according to their therapeutic history, the animals were classified as: patients which did not receive antibiotic treatment - control group, patients which received antibiotic more than 90 days before the diagnosis of urinary stones - group 1, and patients which received antibiotics less than 90 days before the diagnosis of urinary stones - group 2 (Table 1).

Table 1

The distribution of patients according to urolithiasis type and antibiotic therapy

Groups	Dogs			Cats		
	Stones	Sediment	Total	Stones	Sediment	Total
Control	8	1	9	4	9	13
Group 1	3	1	4	5	4	9
Group 2	6	5	11	16	9	25
Total	17	7	24	25	22	47

2). The Chi-Square test was performed to analyze the highlighted data (Table

Table 2

Chi-Square Tests

	Dogs			Cats		
	Value	df	Asymp. Sig. (2-sided)	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.866 ^a	2	.239	3.818 ^a	2	.148
Likelihood Ratio	3.039	2	.219	3.880	2	.144
Linear-by-Linear Association	2.730	1	.098	3.550	1	.060
N of Valid Cases	24			47		

Dogs: a - 4 cells (66.7%) have expected count less than 5. The minimum expected count is 1.17.
Cats: a - 2 cells (33.3%) have expected count less than 5. The minimum expected count is 4.21.

Thus, in dogs, there were no significant differences in urinary stones and sediment formation related to antibiotic therapy ($\chi^2 = 2.866$, $p = 0.239 > 0.05$). No significant differences were found in cats either ($\chi^2 = 3.818$, $p = 0.148 > 0.05$).

The results obtained through data processing were compared to literature data. Thus, Houston and Moore (11) showed that 78% of the 50,000 uroliths being studied came from dogs and 22% from cats. Picavet et al. (17) reported a urolith prevalence rate of 65% in dogs and 35% in cats. However, the prevalence of cases in the Timisoara veterinary clinic was higher for cats (60%).

Regarding location, the identification of uroliths mainly in the bladder (87%) is consistent with the data presented by Hesse et al. (10) and Picavet et al. (17). Picavet et al. (17) underlined that 78% of canine uroliths and 81% of feline uroliths originated from the bladder. However, this finding can be attributed to some extent to the anatomical differences between species, regarding the positioning of the kidneys and bladder in relation to gravity in quadrupeds and bipeds (Markwell et al., 2000, cit. by 19).

Although Picavet et al. (17) showed that, in dogs, 71% of struvite stones were diagnosed in females and 60% of calcium oxalates in males; struvite stones were the only type identified in the current study, mainly in males (83.78%).

Okafor et al. (16) stated that small breeds are more likely to develop urolithiasis with struvite stones compared to medium and large breeds. Houston and Moore (12) had the same results. On the other hand, Brandenberger-Schenk et al. obtained the highest prevalence of urolithiasis in mixed-breed dogs (4). Picavet et al. (17) showed that the most affected breeds were Bichon Frise, Schnauzer, Shih-Tzu, Dalmatian, Yorkshire terrier and Poodle, and an increased percentage of uroliths (56%) in small breeds. Urolithiasis was diagnosed mainly in small breed dogs (Shih-tzu, Pug, Cocker Spaniel, Bichon Maltese) in the current study as well.

Houston et al. (11, 12) showed that, in cats, the uroliths are mostly identified in mixed-breed felines. Hesse et al. (10) obtained a 64% rate, and Picavet et al. (17) reported a 77% rate of uroliths in mixed-breed cats. The study conducted at the Timisoara clinic showed that 88% of the affected cats were mixed-breed, in accordance with literature data.

The results obtained by Picavet et al. (17) show that in 7% of dogs and 8% of cats the disease relapsed. In this study, the recurrence rate was much higher for both dogs and cats (33% and 19%, respectively). One factor that may be involved in the recurrence of urolithiasis could be the effective dietary preventive strategy.

During the study, it was observed that a high percentage of dogs had received antibiotic therapy prior to the diagnosis of urolithiasis (1).

Cases of crystalluria caused by amoxicillin have been reported in human geriatric patients. Apparently, intravenous treatment with high doses correlated with acidic urinary pH and reduced water intake lead to renal failure and crystalluria (6, 9, 21). Considering that amoxicillin was the most commonly used antibiotic in the Timisoara clinic, the influence of antibiotic therapy on urolithiasis was calculated. Thus, by performing a statistical test for the studied groups, there were no significant differences in the appearance of urinary stones and sediment, in relation to antibiotic use.

Conclusions

Following the evaluation of 114 urolithiasis patients (46 dogs and 68 cats), no statistically significant results were obtained regarding the influence of breed, sex or age on the occurrence of urolithiasis.

However, it is worth mentioning that 71% of affected dogs were small breed dogs, respectively, Shi-Tzu and Pugs, and 88% of cats with urolithiasis were mixed-breed. Also, 85% of cats with urolithiasis were males, whereas in dogs, both sexes were equally affected. In cats, most patients were in the age range of 2 to 4 years, and in dogs, the most affected age group was between 6 and 8 years. The most common location of urolithiasis in both dogs and cats was the bladder, and the only type of diagnosed stones were struvite stones.

The urolithiasis recurrence rate was higher than most data reported in literature, which should be a signal for a more laborious investigation of the etiopathogenesis for each patient, in order to achieve a more effective therapeutic management.

Based on the recorded data, it is impossible to establish a significant influence of antibiotic therapy on the presence of urolithiasis.

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PARACLINICAL INVESTIGATIONS ON PROTEIN METABOLISM IN DAIRY COWS

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Summary

Throughout the complex and multiple functions, proteins in the animal body act decisively in ensuring its' vital processes, in reproduction and in the production of meat, eggs, milk and wool. The body's protein needs are provided by the food ratio, with certain particularities occurring depending on the species. All above considered, throughout this paper we set out to investigate the proteinogram of dairy cows according to the physiological state of each cow. The research was carried out on two categories of dairy cows, both categories clinically healthy and aged between 3 to 7 years. The first category which was appreciated as the control group included cows that are not pregnant. The second category was considered as the experimental group and included cows that were in their gestation period. The cattle belong to a private sector farm in Mehedinți county. Blood was collected from both categories of dairy cows starting with the 2nd month of gestation until the 9th month. The determination of protein fractions was performed by electrophoresis. The values of albumins, globulins, total proteins and globulin fractions from both categories of bovines were statistically processed by the nonparametric Mann – Whitney test. Throughout the research it was found that the cows from the control group have much smaller values with reference to total proteins than the cows from the experimental group. During the gestation period the values of albumins oscillates, reaching the maximum value in the 6th month, then the values of the albumins decrease until the 4th month, approaching the values we recorded from the cows in the control group. α -globulins grow significantly in pregnant cows starting with the 5th month of gestation, reaching maximum values in the 8th month, and then the values keep a constant limit. β -globulins values oscillate between high values in the 8th month and small values in the 5th month of gestation. γ -globulins reach a maximum value in the 8th month of gestation and then the values decrease near parturition, nearing the values obtained from the cows in the control group.

Keywords: dairy cows, paraclinical investigations, protein metabolism, proteinogram

Proteins in the animal body, throughout the complex and multiple functions act decisively in ensuring its' vital processes, in reproduction and animal production. The body's protein needs are provided by the food ratio, with certain particularities occurring depending on the species (4, 6).

Therefore the ruminal microorganisms are capable of protein synthesis from non-protein nitrogen. Protein deficiency is observed in cows through: reduction of hepatic albumin biosynthesis, prothrombin and fibrinogen with decreased total serum proteins (especially albumin), reducing appetite by decreasing the synthesis of digestive enzymes. This aggravates the energy deficit and protein deficiency of

the body causing disturbances in the processes of hematopoiesis and leukopoiesis which clinically manifests itself in the form of anemia. It also decreases the formation of anterohypophyseal gonadotropins and sex hormones causing sterility and disruption of osteogenesis with the clinical occurrence of osteoporosis (2, 5, 7).

All above considered, throughout this paper we set out to investigate the proteinogram of dairy cows according to the physiological state of each cow.

Materials and methods

The research was carried on a number of 35 Romanian puddle cows which were divided in 2 categories, both clinically healthy and aged between 3 to 7 years. The first category which was appreciated as the control group included 5 non-pregnant cows. The second category was considered as the experimental group and included 30 cows that are in their gestation period. The cattle belong to a private sector farm in Mehedinți county.

The cows were divided into 7 batches (of 5 cows each) as follows:

Batch I: 5 non-pregnant cows;

Batch II: 5 pregnant cows in the 4th month of gestation;

Batch III: 5 pregnant cows in the 5th month of gestation;

Batch IV: 5 pregnant cows in the 6th month of gestation;

Batch V: 5 pregnant cows in the 7th month of gestation;

Batch VI: 5 pregnant cows in the 8th month of gestation;

Batch VII: 5 pregnant cows in the 9th month of gestation.

Blood samples were taken from the jugular vein without anticoagulant (EDTA) for laboratory biochemical examination. The samples were stored at room temperature for 2-5 hours to cause blood clotting and then centrifuged at 1200 rpm for 10 minutes. The determination of protein fractions were performed by electrophoresis, having as principle the property of fractions to migrate in an electric field inversely proportional to their molecular weight.

Results and discussions

The values of proteins, albumin, globulins and globulin fractions (%) from both categories of bovines were statistically processed by the nonparametric Mann-Whitney test being able to be appreciated in Table 1.

It is noted that cows that are not in their gestation period have the lowest protein value. Starting with the 5th month of gestation total proteins begin to increase progressively until the 8th month after which there is a stagnation until parturition. In the 9th month there is a decrease in protein concentration due to its passage into colostrum milk (1, 3, 8).

In the case of albumin, there is a significant difference between the two categories discussed. During gestation, marked oscillations are observed with reaching the maximum threshold in the 6th month of gestation. In the 7th month of

gestation, the albumin registers a value (30.2 ± 1.56) close to that of non-pregnant cows (29.0 ± 0.40) after which it returns to average values in the 9th month (32.0 ± 2.20).

Table 1
Concentration of total proteins and fractionated proteins in the cows taken into the study

Month of gestation	Total proteins (g/l)	Albumins (g/l)	Globulins (g/l)	α (g/l)	β (g/l)	γ (g/l)	A/G (g/l)
Non-pregnant	65.6 \pm 1.32	29.0 \pm 0.40	36.6 \pm 1.40	7.46 \pm 0.55	8.18 \pm 0.42	19.61 \pm 1.71	0.80 \pm 0.04
IV	70.2 \pm 2.96	32.4 \pm 3.50	39.6 \pm 3.68	8.73 \pm 0.20	8.54 \pm 0.89	22.37 \pm 4.02	0.80 \pm 0.14
V	71.1 \pm 3.12	32.0 \pm 2.00	38.2 \pm 3.52	8.15 \pm 1.57	6.21 \pm 0.94	20.94 \pm 2.67	0.90 \pm 0.12
VI	72.1 \pm 1.60	33.3 \pm 1.70	37.8 \pm 3.68	9.33 \pm 0.93	6.65 \pm 1.08	19.69 \pm 2.85	0.90 \pm 0.13
VII	74.8 \pm 3.48	30.2 \pm 1.56	42.6 \pm 5.60	11.56 \pm 1.96	7.74 \pm 1.05	24.23 \pm 3.44	0.70 \pm 0.11
VIII	75.5 \pm 4.50	31.5 \pm 1.70	44.0 \pm 5.20	12.34 \pm 2.57	8.58 \pm 1.05	23.49 \pm 3.53	0.70 \pm 0.12
IX	69.0 \pm 3.80	32.0 \pm 2.20	36.5 \pm 3.70	7.61 \pm 0.81	7.74 \pm 1.57	19.22 \pm 2.40	0.90 \pm 0.12

The value of globulins varies depending on the limits of the gestation.

The maximum value of globulins (44.0 ± 5.20) is observed in the 8th month of gestation, after which there is a significant decrease close to the value recorded in the category of non-pregnant cows (36.6 ± 1.40).

With regard to α -globulins in non-pregnant cows, low values are recorded (7.46 ± 0.55).

Starting with the 5th month and with the advancement of the gestation there is a marked increase in the α -globulins (8.15 ± 1.57), reaching the maximum level in the 8th month (12.34 ± 2.57).

There are no major differences between the two categories studied for β -globulins. Starting with the 5th month of gestation, a decrease is observed (6.21 ± 0.94) then a significant increase with reaching the peak in the 8th month (8.58 ± 1.05).

In the 9th month of gestation β -globulin level registers an average value (7.74 ± 1.57) similar to the value registered in the 7th month of gestation (7.74 ± 1.05).

Starting with the 4th month of gestation, there is an increase in the value of γ -globulins (22.37 ± 4.02), after which there is a decrease in the 6th month (19.69 ± 2.85), following that in the 7th month to register the maximum value (24.23 ± 3.44). In the 9th month of gestation γ -globulins register the lowest value (19.22 ± 2.40) being close to the one registered from the first category of non-pregnant cows (19.61 ± 1.71).

The 8th and 9th month of gestation were analyzed comparatively because they have a special significance in the preparation of the animal for parturition. The maximum value of total proteins is recorded in the 8th month of gestation (75.5 ± 4.50) compared to the ones in the 9th month that register a lower value (69.0 ± 3.80).

The results signify a visible increase in the concentration of total serum proteins, globulins, α -globulins and γ -globulins in the 8th month of gestation and a visible decrease in the 9th month of gestation.

The average values of the concentration of serum proteins and those fractionated individually from the serum of non-pregnant cows are low compared to the values obtained in pregnant cows in the 8th month.

Cows that are in the 9th month of gestation have the lowest values in all the parameters taken into consideration and in the 8th month they reach the maximum concentration.

The value of fractionated proteins is significantly low in the 9th month of gestation, being close to the value of non-pregnant cows.

Conclusions

Non-pregnant cows have the lowest value of serum proteins compared to pregnant cows.

In pregnant cows, total serum proteins increase progressively until the 8th month of gestation and significantly decrease in the 9th month of gestation.

During the gestation there are oscillations of albuminemia, reaching the maximum threshold in the 6th month of gestation, whereas in the 7th month the value is close to the value of non-pregnant cows.

The globulin maximum value is noticed in the 8th month of gestation, followed by a marked decrease.

Starting with the 5th month of gestation, as the gestation progresses there is a marked increase of α -globulins, reaching the maximum value in the 8th month of gestation. Following this, the value returns to a level close to the one of non-pregnant cows.

Starting with the 5th month of gestation, it was noticed that there was a decrease in β -globulins, followed by a marked increase and reaching the maximum threshold in the 8th month of gestation. In the 9th month of gestation the average value of β -globulins is close to the value noticed in the 7th month of gestation.

In the 7th month of gestation, γ -globulins reach the maximum value. In the 9th month of gestation γ -globulins have the lowest value, being close to the value of non-pregnant cows.

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DIAGNOSTIC CHALLENGES IN *LEISHMANIA INFANTUM* INFECTION

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Summary

Canine leishmaniosis (CanL) is a zoonotic parasitic disease caused by *Leishmania infantum* in the Mediterranean area and transmitted by phlebotomine sand fly vectors. The domestic dog is the main reservoir host. Clinical manifestations are highly variable, ranging from subclinical infections to generalized disorders. Diagnosis is performed on the basis of clinicopathological manifestations and by confirmation of infection using mainly serological and molecular techniques. The aim of this study was to report four cases dog diagnosed in central Italy, and infected with *Leishmania infantum*, and to emphasize the differences between clinical forms and stages of the disease.

Keywords: leishmaniosis, phlebotomies, diagnostic challenges

Leishmaniosis is a chronic and severe zoonotic disease with a long incubation period in which clinical signs develop within months to years after the initial infection. Among the 53 species recognized worldwide, 20 seems to be also infective to humans. Different parasite species are not differentiated by morphological differences, but rather based on geographical, biological, and clinical features (9, 10).

Leishmania infantum has been identified as the main etiologic agent of canine leishmaniosis (CanL), which is a major global zoonosis that is potentially fatal to humans and dogs, and it is one of the world's most important emerging diseases (16). *Leishmania infantum* is a protozoon characterized by the possession of a kinetoplast, a unique structure formed by massed DNA (circles or lattice) within a single large mitochondrion strongly associated with the flagellar basal body (2).

The infestation is arthropod vectors needed, and sand fly female *Phlebotomes* are the principal way of transmission: *P. perniciosus*, *P. neglectus*, *P. ariasi* and more. These neglected diseases are prevalent in at least 98 countries and 3 territories on 5 continents, of which the majority are underdeveloped countries. Approximately 12 million people are infected with a species of *Leishmania* at any given time (16).

Dogs are the main reservoir of infection, and in some disease-endemic areas seroprevalence of canine leishmaniosis is more than 30% (8). In the last years, the geographic distribution of canine leishmaniosis has spread, and new foci of disease have been reported in countries such as the United States (7, 14). Global warming

is a possible cause of the spread of the disease to cooler areas, and the increased movement of infected animals from areas where the disease has traditionally been endemic can, with the spread of sand fly vectors, facilitate this process (7).

In Europe, both canine leishmaniosis and human visceral leishmaniosis are endemic in Mediterranean areas characterized by a dry, hot summer and mild winter temperatures (8).

However, foci of canine leishmaniosis have never been reported in continental climate regions, which are characterized by large seasonal temperature changes between hot summer and cold winters. Until recently, stable endemic foci of both human visceral leishmaniosis and canine leishmaniosis have been present only in southern, central, and insular regions in Italy. However, new foci of canine leishmaniosis and the presence of competent sand fly vectors have also been reported in northern regions of the country, where autochthonous cases had not been reported previously (8).

The course of infection may be different from one individual dog to another, ranging from spontaneous cure to acute evolution that leads to death, if proper management and therapy are not adopted (16). Four main forms of leishmaniosis are currently identified: cutaneous, muco-cutaneous, visceral, and asymptomatic one and four stages of severity are classified. More than 80% of dogs with leishmaniosis disease develop mainly dermatological signs and in some cases that could be the only clinical manifestation of the disease.

Leishmania spp. produce lesions throughout the body of the host, especially in the lymph nodes, liver, spleen, and skin, where the proliferative inflammatory infiltrate is dominated by macrophages. *Leishmania spp.* also induce immune-complexes deposits in some tissues (2, 4, 12, 13).

Clinical manifestations are highly variable, ranging from subclinical infections to generalized disorders characterized by fever, weight loss, generalized lymphadenopathy, splenomegaly, ulcerative, exfoliative or nodular skin lesions, onychogryphosis, ocular lesions, epistaxis, lameness and polyuria, and polydipsia (6, 15, 17).

Typical laboratory abnormalities are polyclonal hyperglobulinaemia and hypalbuminaemia, non-regenerative anemia, leucocytosis or leukopenia, thrombocytopenia or thrombocytopenia, and abnormality of hepatic or renal profile (18). The anemia and dysproteinaemia are considered the main laboratory findings (16).

Clinical diagnosis is relatively easy in patients showing multiple typical signs, especially when the animal lives in or has spent time in an endemic area. However, if the dog presents only one symptom or lesion, diagnosis may become more difficult.

As well, the diagnosis may be difficult when appear unusual clinical forms, for instance associated with specific skin lesions, oral lesions, chronic colitis, hemostatic problems and/or disorders of the cardiovascular, respiratory, and Musculo-skeletal system (5, 15).

The diagnosis is made considering the epidemiological origin and the set of clinical signs presented by the dog. Due to the large number of asymptomatic dogs and the absence of pathognomonic clinical signs, the diagnosis depends on laboratory support. All the parasitological, immunological, and molecular techniques available for diagnosis are important and need to be interpreted according to their benefits and limitations (16, 17, 19).

The aim of this study was to report four cases dog diagnosed in central Italy, and infected by *Leishmania infantum*, and to emphasize the differences between clinical forms and stages of the disease.

Materials and methods

The study was conducted in the private Vet Clinic from Lazio area located in the central Italy.

The first case report is represented by a mix- breed male dog, fifteen years old (Fig. 1):

- Clinical Signs: None.
- Skin Lesions: None.
- Diagnosis: The dog was visited for a suspected anemia, once established the CBC and the biochemistry profile immunofluorescence analysis was conducted.
- Treatment: Supportive treatment aimed at improving the general condition of the dog.

The second case report is represented by dachshund three years old dog that presents (Fig. 2):

- Clinical Signs: General physical examination: lethargy, moderate lymphadenopathy.
- Skin Lesions: Exfoliative dermatitis on the top on the head, papular dermatitis on the inner pinnae, eyelids, bridge of the nose and lips.
- Diagnosis: Cytology revealed macrophagic inflammation with some neutrophils. Nodular to diffuse pyogranulomatous inflammation on skin biopsies with positive *Leishmania*-specific immunohistochemistry.
- Treatment: Miltefosine 2mg/kg SID for 28 days; Allopurinol 10mg/kg BID for 28 days.



Fig. 1. Case 1



Fig. 2. Case 2

The third case report is represented by a mix-breed dog, six years old in which the positivity of the infection was previously confirmed (Fig. 3).

- Clinical signs: Fever, vomiting, diarrhea, mucous membrane pallor, cutaneous ulcerative dermatitis in particular on the nose and ears, cachexia, lethargy, onicogriphosis, epistaxis.

- Skin lesions: Keratinization disorders, papular dermatitis on the region of the nose and mouth (snout). Periorbital region is compromised by desquamative dermatitis, granulomatous lesions.

- Diagnosis: Based on clinical signs and blood test analysis, urine analysis.
- Treatment: Deflamon 40 ml, Synulox 2 ml, Benazepril 0.25mg/kg, Allopurinol 10mg/kg, Prednisolone 0.5 mg/kg for 28 days.



Fig. 3. Case 3

- The last case report is represented by a female dog, five years old (Fig.4):
- Clinical signs: BCS 3/6, fever, lymphadenomegaly, popliteal and axillar lymphnodes compromised, dandruff, loss of body weight.
 - Skin Lesions: Focal dermatologic nodular lesion on sub zygomatic region, mild exfoliative dermatitis on the periocular region.
 - Diagnosis: CBC mild non-regenerative anemia, mild leucocytosis and monocytosis, ALB/GLB 0,5, FNA from the nodular lesion.
 - Treatment: Allopurinol 10 mg/kg and Miltefosine 2 mg/kg SID for 28 days; dietary renal treatment (HPV); N-meglumine antimoniate and allopurinol lower dose. Inj at different sites every 3 days.



Fig. 4. Case 4

Results and discussions

✓Case 1:

The first case report after all research done resulted in an asymptomatic patient.

✓Case 2:

The second case report after obvious injuries and laboratory tests resulted a cutaneous form of Leishmaniosis.

✓Case 3:

The third case report describes a mucocutaneous form of Leishmaniosis.

✓Case 4:

The last case report resulted in the worst form of *Leishmania* infection, the visceral stage. Unfortunately, the patient died after 10 days of treatment because of the general conditions, which leads to the collapse and Multiple Organ Dysfunction Syndrome (MODS).

Ferroglio E. et al. (8) reported that canine leishmaniosis is currently expanding in continental climate areas of northwestern Italy, far from the recognized disease-endemic areas along the Mediterranean coasts. Based on similarities in climate and major landscape features of this study area, the spread of canine leishmaniosis to other regions of central Europe can be foreseen soon.

A study conducted in northern Italy concluded that this area is now focally endemic for visceral leishmaniosis (VL) and that a moderate risk of human disease does exist, although the current intensity of *Leishmania* transmission seems to be lower than in traditional settings of Mediterranean VL. Travelers from *Leishmania*-free countries should be made aware of the new situation and advised to protect themselves and their pets from sandfly bites during summer holidays in hilly and low-range mountain zones of northern continental Italy (11).

An epidemiological survey of canine leishmaniosis (CanL) was performed during a 3-year period in a public doghouse of the Bologna province. The incidence values ranged between 4.9% and 6.6%, indicating a stable focus of leishmaniosis. The entomological survey, performed by sticky and CO₂-baited traps in 2008, showed the vector *Phlebotomus perfiliewi*. This study identified a stable focus of CanL in an area that was not considered eco-compatible with the presence of the vector and infection. The results confirm the northward spread of CanL toward areas not previously affected by autochthonous foci (3).

A cutaneous involvement in canine leishmaniosis, caused by *Leishmania infantum*, is the most frequent clinical manifestation of the zoonotic infectious disease described in Iran. A 4-month-old female Shih Tzu-terrier dog with significant weight loss and depression and chronic erosive skin lesions around eyes and the area above the nose. The main clinicopathological findings included marked leucocytosis, neutrophilia, left shift, monocities, mild hypoproteinaemia, and hypoalbuminemia. The diagnosis of leishmaniosis was performed based on the presence of many *Leishmania* amastigotes in skin Fine Needle Aspiration (FNA) (1).

Another study highlights a canine visceral leishmaniosis case diagnosed in Londrina, an indene city, and its investigation. A street animal with extensive dermatological lesions, onychogryphosis, mild anemia and leukopenia was attended at a veterinary hospital in Londrina, where positivity was reported for *Leishmania* spp. in serological tests. Cytology was positive in bone marrow, PCR and parasite cultures were positive in the skin, spleen, liver, lymph node and bone marrow, and DNA sequencing confirmed the species of the parasite as *L. (L.) infantum* (6).

The epidemiological, clinical, and laboratory aspects of Can-L are very variable, which makes it difficult for veterinary practitioners to complete a diagnosis and then treat and control the disease, especially due to the lack of more effective drugs and vaccines. However, considerable efforts are being made by professionals from multidisciplinary areas to improve the knowledge about this parasitic disease, so that prevention, treatment, and control may be improved in the future (16).

Conclusions

The complexity of CanL and the wide range of its clinical manifestations, from inapparent infection to severe disease, make the management of CanL challenging.

Diagnosis is performed on the basis of clinicopathological manifestations and by confirmation of infection using mainly serological and molecular techniques. A staging system that divides the disease into four stages is aimed at assisting the clinician in determining the appropriate therapy, forecasting the prognosis, and implementing follow-up steps required for managing the leishmaniosis patient.

Prevention should be an integrated approach including vaccination against *L. infantum* with an effective canine vaccine and topical insecticide application.

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RESEARCH ON THE EVOLUTION OF AFRICAN SWINE FEVER IN ARAD COUNTY IN 2019

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Summary

African Swine Fever (ASF), is caused by the ASF virus, a member of the *Asfarviridae* family. It is one of the most important diseases in the pig breeding industry. The disease produces very high losses having a major economic impact in the pig breeding industry. It is a disease listed on the OIE as a notifiable disease. Due to the national and international trade restrictions imposed by this disease, very significant losses occur. The disease affects domestic and wild pigs, being receptive to all age categories and all breeds of pigs. The paper's aim was to monitored the evolution of African Swine Fever in 2019, in the populations of domestic and wild pigs in Arad County. ASF was diagnosed in domestic pigs in Arad County in 35 outbreaks located in 10 localities, including Arad. In wild boars, 59 cases were identified from wild boars found dead or from hunted wild boars.

Keywords: African Swine Fever, domestic pigs, epidemiology

ASF is one of the most important diseases, perhaps the most important from an economic point of view, due to the national and international restrictions that are imposed, along with the suspicion and confirmation of this disease (1, 2, 5). The disease was first reported in 1921 in East Africa. Since then and until today there is a lot of information and reports at international and national level, but still there are not enough good prevention and control measures to stop the appearance and evolution of the disease. Until now, there is no treatment or vaccine available on the market.

ASF is evolving rapidly, with high mortality, being considered an extremely contagious disease. It is a notifiable disease, it is not a zoonosis and it has no impact on human health.

By quickly detecting clinical signs by veterinarians and by confirming the diagnosis through laboratory tests, followed by the application of measures to minimize the spread of the virus, this disease can be controlled.

African Swine Fever (ASF), is caused by the ASF virus, a member of the *Asfarviridae* family.

It was first reported in East Africa in the early 1900s in domestic pigs (*Sus scrofa domesticus*) and warthogs (*Phacochoerus africanus*), and the latter were quickly identified as a possible source of the domestic infection (1, 3, 4).

Materials and methods

The present research was performed on the existing pig population in farms and households in Arad County. In order to obtain the primary data on the epidemiological situation of AFS for 2019, we benefited from the primary records of the Sanitary Veterinary and Food Safety Directorate of Arad. Along with the primary data, the following were studied: the analysis bulletins issued following the laboratory examinations, the results of the epidemiological investigations, the sanitation plans and the records of the epidemiology department within SVFSD Arad.

We mention that in the records of the SVFSD there were no data on the evolution of African Swine Fever until 2019, because this disease was not confirmed or suspected in this county.

We also specify that all suspicious samples from suspicions were sent to the Institute of Diagnosis and Animal Health (IDSA) Bucharest.

Results and discussions

The research is extended during 2019 and refers to the population of domestic and wild pigs in Arad County. The primary data were processed and interpreted, and the significant results were retained and are summarized in the paper.

In 2019, in Arad County, the situation of ASF in domestic pigs from non-professional farms is presented according to the data in Table 1.

Table 1

The situation of African Swine Fever in domestic pigs from non-professional farms in Arad County, in 2019

No.	Locality	No of positive cases	No of dead pigs	No of killed pigs
1.	SÂNPETRU- GERMAN	5	4	50
2.	ZĂDĂRENI	2	0	2
3.	FELNAC	2	1	30
4.	CINTEI	7	6	53
5.	PECICA	4	7	71
6.	NĂDAB	9	10	148
7.	ȚIPAR	1	1	5
8.	MUNAR	2	1	5
9.	CĂLUGĂRENI	1	1	3
10.	ARAD	2	1	101
Total		35	32	468

During the study period in Arad County, 35 outbreaks of African Swine Fever were diagnosed in domestic pigs from non-professional farms, located in 10 localities (including Arad). Following the declaration of the disease in these localities, it was necessary to apply control measures, measures that required, in the first stage, the killing of 468 pigs in the infected localities.

The anti-epizootic department decided based on the epidemiological situation in Arad county, that in the localities Sânpetru-German, Cinteii and Nădab apply the measure of preventive killing of all pigs in these infected localities, located near commercial farms. The distribution of the number of pigs killed in each locality, as a result of the application of the preventive killing method, is shown in Table 2.

Table 2

Distribution of killed pigs as a result of the applying of the preventive killing method

Date of application of the measure	Locality	Number of households	Number of killed pigs
06.08.2019	SÂNPETRU- GERMAN	6	39
23.08-25.10.2019	CINTEI	107	1156
26.08-10.09.2019	NĂDAB	180	1577
End 13.09.2019	Total	292	2760

From data presented in Table 2 it results that in order to prevent the transmission of the infection to the pigs from the commercial pig farms it was necessary to kill, in the contaminated localities, another 2760 pigs. Although this measure was very expensive, it gave results, considering the fact that, later, the disease in Arad County did not penetrate the commercial pig farms.

In total, in Arad County, in 2019, 3228 pigs (2760 + 468) were killed to control African Swine Fever in domestic pigs.

Following the diagnosis of African Swine Fever in domestic pigs from non-professional farms in Arad County in 2019, control measures were applied that allowed the extinction of the disease according to the data in Table 3.

Following the application of measures to combat African Swine Fever in domestic pigs from non-professional farms in Arad County in 2019, it was possible to extinguish the disease starting with 05.11.2019, a process that was completed at county level on 29.11.2019. The period of time in which the outbreaks were considered active in the localities declared infected was between 59 and 135 days. It should also be mentioned that in Arad in January 2019, an outbreak of disease was registered as a result of the clandestine trade with young pigs, an outbreak that was extinguished 70 days after the declaration date (09.04.2019).

Table 3

Situation of extinction of African Swine Fever outbreaks in domestic pigs on non-professional farms

No.	Locality	Number of outbreaks	Declaration date	Extinction date	Duration (days)
1.	SĂNPETRU- GERMAN	5	27.07.2019	29.11.2019	135
2.	ZĂDĂRENI	2	10.09.2019	29.11.2019	80
3.	FELNAC	2	07.08.2019	29.11.2019	114
4.	CINTEI	7	12.08.2019	05.11.2019	69
5.	PECICA	4	19.08.2019	29.11.2019	64
6.	NĂDAB	9	21.08.2019	05.11.2019	75
7.	ȚIPAR	1	28.08.2019	05.11.2019	68
8.	MUNAR	2	04.09.2019	29.11.2019	71
9.	CĂLUGĂRENI	1	11.09.2019	29.11.2019	79
10.	ARAD	1	01.10.2019	29.11.2019	59
			29.01.2019	09.04. 2019	70
	Total	35			

We consider, like other researchers in Europe, that the virus was introduced into pig farms in an indirect way, by non-compliance with biosecurity rules and zoo sanitary measures applied on farms (4, 7).

Following the epidemiological investigations carried out in these farms, it was found that the main source of infection on the farm was represented by dead wild boars, dead in the vicinity of the farm (about 10 km). Subsequently, disease outbreaks were also confirmed in wild boar populations in the vicinity of households where the disease was confirmed in domestic pigs, as was the case in Ceala and Sanpetru German (Tables 1 and 4). These observations have also been found by other researchers (5).

The situation of African Swine Fever in wild boars in Arad County is presented in Table 4. As a result of the application of the Veterinary Sanitary Surveillance Program for African Swine Fever, controls were carried out in hunting grounds in Arad County, to detect the disease initially only in wild boar carcasses, according to the data in Table 4.

As shown in Table 4, samples from 14 wild boar carcasses were sent to confirm African Swine Fever, of which 11 carcasses were confirmed.

We consider, like other authors, that the risk factors for the spread of ASF are represented by free wild boars circulating near or in the vicinity of pig farms, households, illegal movement of pigs from contaminated areas to disease-free areas, illegal trade of animal products and by products from positive animals, non-compliance with procedures and biosecurity measures (4).

Table 4

PPA suspicions - cases (wild boars found dead)

Hunting fund	Suspicion	Wild boars found dead	Wild boars found dead and unconfirmed	Confirmed dead wild boars	Dead wild boars from which no sample was collected
HF- 11 CEALA	0	4	0	4	0
HF- 5 SÂNPETRU GERMAN	0	9	2	7	0
HF- 58 RĂDEȘTI	0	1	1	0	0
AR Total	0	14	3	11	0

Following the detection of African Swine Fever in the forest environment, the County Anti-Epidemic Command ordered the hunting of all hunting funds in Arad County.

A number of 57 hunting funds were studied and supervised, of which, out of 2585 hunted wild boars; samples were sent to confirm the African Swine Fever, of which only 48 carcasses were confirmed. These confirmed cases came from the Sânpetru German hunting fund, 31 confirmed cases, out of 66 wild boars hunted; from the Ceala fund there were 75 hunted wild boars, of which 16 cases were confirmed and 59 cases unconfirmed; and from the Mailat hunting fund there was only one case hunted and that was confirmed as positive. Cumulating these cases, with the 11 positive ones from the dead wild boars, it results that in Arad county, in 2019, 59 wild boar with African Swine Fever were detected. From these hunting funds, 2537 wild boars were hunted and unconfirmed.

In Poland it has been shown that cases of ASF have been found where the density of wild boars has been higher than 0.4-0.5 animals / km² (4).

In Latvia, the persistence of infection in wild boar populations is considered to be due to the long-term resistance of the virus to the environment, implicitly to corpses that remain in the field for a longer period of time. However, studies and research are still needed to better understand the role of habitat and carcasses in the strategy of virus survival in the environment (4).

The territorial distribution of the origin of positive pigs for African Swine Fever was the Mureș river meadow, in the vicinity of Pecica commune, and the second area would be the area of Nădab locality.

From the sanitary veterinary documents it results that for the control of African Swine Fever in Arad county in 2019, compensations were paid for the 326 affected farms amounting to 1,863,500 lei (6).

Conclusions

In 2019, in the pig population in Arad County, investigations were carried out regarding African Swine Fever, following by declared of several outbreaks of disease, both in pigs from households and in wild boars.

African Swine Fever was diagnosed in domestic pigs in Arad County, in 35 outbreaks located in 10 localities, including Arad. In wild boars, 59 cases were identified from wild boars found dead or from hunted wild boars.

In order to protect commercial pig farms, the County Anti-Epidemic Command ordered the preventive killing of 2,760 pigs, which would stop the transmission of the disease in these farms.

As a result of the application of drastic measures to combat the outbreaks of African Swine Fever in Arad County, from 2019, they could be closed at the end of the year, more precisely on 11/29/2019.

For the control of African Swine Fever in Arad County, in 2019, 1,863,500 lei were spent from the state budget.

Considering the territorial distribution of African Swine Fever, in the vicinity of commercial pig farms in Arad County, we consider that in the future in this county epidemiological vigilance must be strengthened.

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CURRENT INFORMATION ON SARS-COV-2 IN PETS

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Summary

SARS-CoV-2 is the cause of coronavirus disease 2019 (COVID-19) and responsible for the current global pandemic. An amplified zoonotic transmission event in a seafood and animal market in Wuhan (China), is suspected to be the site of the first significant infectious epidemic in humans, with bats and / or pangolins speculated as the potential species of origin based on the sequence homology of coronaviruses isolated from these animals. The close association between humans and pets raises concerns about the potential risks of transmitting SARS-CoV-2 from humans to animals ("reverse zoonosis"), and the role that infected animals may play in the spread of the disease (such as becoming a new reservoir for the virus). Further research on SARS-CoV-2 infection in various animal species is needed in order to identify susceptible hosts and to better understand the infection, disease, clinical course and transmission capacities of sensitive animal species. This knowledge is important for risk assessment, implementation of mitigation strategies, resolution of animal welfare issues, and development of preclinical animal models for the evaluation of drug and vaccine candidates for COVID-19.

Keywords: virus, SARS-CoV-2, disease, pets, cats, dogs, infection, transmission

Can SARS-CoV 2 infect dogs?

Following the onset of Covid-19 in humans, several studies have been carried out in pets to determine whether this disease is a risk for our pets and if they could be an intermediate vector.

Dogs like cats are among pets that occupy a large place in human families. Therefore, we will see what the situation is in dogs with respect to exposure to SARS-CoV-2 responsible for COVID-19.

First studies carried out in Hong Kong in March 2020

In Hong Kong, when people suffer from COVID-19, contact cases are placed in quarantine and they have the opportunity to isolate and treat their animals. This is a useful step towards complementing research on COVID-19 in pets.

Screenings were therefore carried out on 15 dogs from households where the owners contracted the disease. 2 dogs were found to be infected with SARS-CoV-2 using numerous tests.

The first dog was a 17-year-old Pomeranian with pre-existing illnesses (heart murmur, systemic and pulmonary hypertension as well as chronic kidney disease). Nasal, oral and rectal swabs were taken. A fecal sample was taken along with a blood sample for serological testing.

Throughout the quarantine period, the dog remained alive with no obvious change in his clinical condition (13).

SARS-CoV-2 RNA was detected from nasal swabs taken from this dog by quantitative PCR (Polymerase Chain Amplification) with reverse transcription (RT-qPCR). Rectal and fecal samples tested negative.

The second dog was a healthy 3-year-old male German Shepherd from another household. The oral and nasal swabs tested positive for SARS-CoV-2 RNA. The rectal samples collected were positive in four of the six tests.

A second dog in this household was sampled four times and tested negative in all tests.

Serum samples taken from dogs in these households were tested for anti-SARS-CoV-2 antibody using 90% plaque reduction neutralization assays. Only the second dog from the second household remained antibody negative.

Viral RNA from nasal samples taken from the 2 dogs that tested positive were sequenced and compared to viruses found in their owners' clinical samples. The viral sequences of the dogs and their human index case from the same household were identical throughout the genome. However, the viruses from the two households were clearly distinguishable (13).

These results demonstrate the infection of these two dogs with SARS-CoV-2. The angiotensin-converting enzyme 2 (ACE2) is known to be the human receptor for SARS-CoV-2, and canine ACE2 is similar to that of humans.

Of the 18 amino acids known to be involved in the interaction between ACE2 and the spike receptor binding domain (RBD) of SARS-CoV-2, five differ between humans and dogs, but none of them they are only found in regions known to disrupt the interaction between the RBD of SARS-CoV and ACE2 (7).

Second studies on cases detected in the United States

The first confirmed case of SARS-CoV-2 infection in a companion dog (German Shepherd) in the United States was located in New York State. Samples from the dog were taken and tested positive.

One of the dog's owners tested positive for COVID-19, and another showed symptom consistent with the virus before the dog showed signs. A second dog in the household showed no signs of illness; however, antibodies have also been identified, suggesting exposure (19).

Human-animal proximity test

Tests were carried out on 21 domestic animals (including 12 dogs) living in close contact with their owners (community of 20 veterinary students) in which 2 students tested positive for COVID-19 and several others showed clinical signs. Although a few canines showed clinical signs indicating coronavirus infection, none of them tested positive and no antibodies were detectable in their blood (by immunoprecipitation test) (14).

These original data are used to establish a better assessment of the host range of SARS-CoV-2 under exposure conditions in the natural environment.

Situation in Italy

The largest study to assess SARS-CoV-2 infection in companion animals was carried out in northern Italy (mainly in Lombardy), on more than 817 animals including 540 dogs living in infected homes (10).

3.4% of dogs (approximately 13 individuals) had neutralizing antibody titers.

Many have tested negative. Note that those from a positive household are more likely to test positive for antibodies.

During this study an observation suggested that a higher proportion of male dogs were HIV positive compared to females. There are clear differences between the sexes, however there appears to be no evidence of a difference in the risk of infection.

In addition, out of 30 puppies under one year old, none tested positive.

Future studies should determine whether this phenomenon is based on physiological or behavioral differences.

Transmission of SARS-CoV 2 in dogs: Transmission between dogs and pets

As we could see in one of the previous studies, the owner of the German Shepherd had a second dog (study in Hong Kong) in which neither the viral RNA nor the antibody responses were detected. Transmission had therefore not occurred between the two dogs sharing the same household.

We have also seen an earlier report that dogs do not replicate the virus in the upper respiratory tract. This is because they do not release viruses after infection, but they seroconvert and develop a response of neutralizing antiviral antibodies (1).

We can conclude that a dog does not pass SARS-CoV 2 to another dog or to a pet.

Transmission between dogs and humans

Studies on dogs from a community of veterinary students infected or showing symptoms, support the evidence of a zero or very low COVID-19 infection rate in these dogs even in a situation of repeated and close contact with these students.

We also know that dogs are currently not considered susceptible hosts to SARS-CoV-2, despite some positive RT-qPCR results in dogs (12).

This suggests that the rate of transmission of SARS-CoV-2 between humans and dogs under natural conditions is still low.

However, the first reports reported in Hong Kong, as well as other similar cases seen elsewhere in the world, show that almost all dogs that have tested positive have come into contact with people with COVID-19.

This is called reverse zoonosis (human-to-animal transmission). It is often possible if infected owners expose their companions to the virus during an acute infection.

In addition, the OIE stated that canids can be infected by being in constant contact with infected people, but that on the other hand there is no evidence defining the role of infected dogs in the spread of SARS-CoV-2 (18).

In conclusion, we represent a much greater risk for them than they are for us (5).

Effects of SARS-CoV-2 in Dogs

Overall, the clinical assessment for this disease includes temperament and assessment for the presence of any clinical signs, including eye discharge, runny nose, ptyalism (excessive secretion of saliva), coughing and sneezing, dyspnea, diarrhea, lethargy, or anorexia. None of the animals showed clinical signs of disease characterized by any of these symptoms at any time during the studies (17).

We observe this situation in the first 2 cases studied in Hong Kong, there were no specific symptoms in either of the infected dogs while they were shedding the virus.

According to another study, infected dogs quickly develop antibodies against viral antigens, but no pulmonary signs or other radiographically detectable abnormalities were noted (11). Likewise, dogs inoculated with SARS-CoV-2 remained clinically normal and afebrile.

In most studies, none of the dogs with neutralizing antibodies showed respiratory symptoms at the time of collection.

Likewise, an experimental infection has shown that in general they develop asymptomatic infections, no clinical disease and excrete low titer or no virus (3).

Finally, so far, all reports of death in infected canine cases have been due solely to diseases or causes other than infection.

Cat infection with sars-cov-2, are cats infected?

A study was conducted on SARS-CoV-2 infection and transmission dynamics in domestic cats (11).

In this study 10 cats were used from 4 to 5 months including 6 cats inoculated with SARS-CoV-2 intranasally and orally (group 1), 2 sentinel cats (group 2) put in contact with the previous 6 one day after their SARS-CoV-2 inoculation and 2 isolated control cats.

For post-mortem examinations 5 cats from group 1 are euthanized: two at 4 days following inoculation (DPC = day post challenge), two at 7 DPC and one at 21 DPC.

The detection of high levels of viral RNA from swab samples and in various organs and tissues, as well as mild to moderate histological changes in the trachea and bronchi associated with viral RNA and viral antigen, and the development of antibodies specific to SARS-CoV-2 demonstrates that cats (infected and contacts) have been productively infected.

Reaction to infection

Symptoms:

Body temperature and clinical signs such as: fever, anorexia, lethargy, respiratory distress, inappetence, depression, cough, sneezing, diarrhea / loose stools, vomiting etc. were recorded daily (11). No notable clinical signs were observed during the study. Cats infected with SARS-CoV-2 therefore appear to be asymptomatic.

At the cellular level:

Overall, no significant changes in most blood cell parameters or serum biochemistry were observed. The white blood cell (WBC) count remained within normal limits for most of the animals during the study. No significant changes were observed in serum biochemical analyzes.

At the pathological level:

Macroscopic pathology of the respiratory tract was examined during the post-mortem examination.

Histologically, pathological changes are limited to the upper and lower respiratory tract (larynx, trachea and main pulmonary, lobar and segmental bronchi) of cats infected with SARS-CoV-2.

Pathological signs are characterized by multifocal lymphocytic and neutrophilic tracheobronchoadenitis of the lamina propria and the submucosa of the trachea and bronchi.

The changes are slight at 4 CPD and moderate at 7 CPD.

The affected submucosal glands and associated ducts were variably distended, lined with attenuated epithelium, containing varying amounts of necrotic cell debris.

No significant pathology was identified elsewhere in the lung parenchyma (bronchioles, pulmonary vessels, alveolar spaces, alveolar septa and visceral pleura) of cats infected with SARS-CoV-2 at 4 and 7 DPC.

No significant histological changes were noted in the airways at 21 CPP, the architecture of the submucosa of the trachea and bronchi being unremarkable and within normal limits.

Body response to infection:

Neutralizing antibodies specific for SARS-CoV-2 were found in sera from main and sentinel cats 10, 14 and 21 days after inoculation. Cats therefore respond to (experimental) infection with SARS-CoV-2 by developing a humoral immune response.

Nasal washes collected at autopsy from all major infected cats examined at 4 and 7 DPC were RNA positive, but negative in the cat evaluated for 21 days later.

No significant histologic changes were noted in the airways at day 21 after inoculation.

The absence of viral RNA shedding and histological changes in the lungs and trachea at 21 days after inoculation suggests that the cats recovered from the infection 3 weeks later.

Replication of the virus in cats

A study was conducted on the replication of SARS-CoV-2 in cats.

7 subadult cats (6 to 9 months old) were inoculated intranasally.

2 animals are euthanized on days 3 and 6 μ l (post infection), respectively, to assess viral replication in their organs.

3 subadult cats were placed in separate cages inside an isolator.

Viral RNA was detected in the nasal turbinate of one animal, as well as in the soft palates, tonsils, tracheae, lungs and small intestine of both animals euthanized on day 3 μ l.

In animals euthanized on day 6 μ l, viral RNA was detected in the nasal turbinates, soft palates and tonsils of both animals; in the trachea of an animal; and in the other's small intestine. However, viral RNA was not detected in any lung samples from either of these animals. The infectious virus was detected in the nasal turbinates, soft palate, tonsils, tracheae and lungs of these cats, but was not recovered in the RNA-positive small intestines. This replication and transmission study is repeated in juvenile cats (aged 70 to 100 days).

Histopathological studies carried out on samples from juvenile cats inoculated with the virus, dead or euthanized on day 3 μ l, revealed massive lesions of the epithelia and lungs of the nasal and tracheal mucosa.

These results indicate that SARS-CoV-2 can replicate efficiently in cats and that younger cats are more vulnerable than older ones.

Transmission of sras-cov-2

The receptor for SARS-CoV and SARS-CoV-2 is ACE2. Protein alignment and phylogenetic analysis of the entire ACE2 protein in mammalian species reveals that, as expected, the monkey ACE2 of macaque (*Macaca mulatta*) and chimpanzee (*Pan troglodytes*) is closest to the Human ACE2 with 94.9 – 99% sequence identity.

Interestingly, of the other mammalian ACE2 sequences analyzed, the following most closely related sequence is from the house cat (*Felis catus*) with 85.2% overall identity to human ACE2 (7).

Cats therefore appear to be susceptible hosts to the human SARS-CoV-2 virus in view of the high degree of similarity between the human and feline forms of the SARS-CoV-2 receptor, ACE2.

However, much remains to be determined about the extent of infection in the feline population, as well as the potential for transmission between cats or to other animals, including humans.

Can cats pass sras-cov-2 to each other?

A study is conducted on 2 groups of cats (2).

In the first group, 3 cats are infected with SARS-CoV-2, and re-infected 28 days after the first inoculation.

In group 2, 2 cats are inoculated as in group 1 and 48 hours after that, 2 naive cats are introduced with the 2 infected cats.

In group 1, all three cats shed the virus orally and nasally for up to 5 days after infection, with maximum titers obtained from nasal shedding on day 3.

Overall, the data demonstrate a clear presence of the infectious virus in the nasal cavity and oropharynx for several days after infection.

In group 2, inoculated cats shed the virus for 5 days after oral and nasal infection, with a pattern similar to that in group 1.

Contact cats, however, shed infectious virus orally 24 hours after exposure and the duration of shedding is prolonged compared to inoculated cats, with peak shedding occurring 7 days after exposure.

The virus was isolated from the trachea, turbinates and esophagus of Group 2 cats autopsied on day 5. No infectious virus was found in the lung or other organs of either cat. This study shows that infected cats transmitted the virus to contact cats, and illustrates how cats shed the virus.

Similar results are found in the 1st experiment presented above.

To find out how cats can become infected with the virus, we look at how the sentinel cats of the first experiment were infected by the main cats (having undergone the inoculation). The main infected cats were able to transmit the virus to

negative contact animals within 2 days of contacting the two groups, since the viral RNA for SARS-CoV-2 is found in them between 2 and 9 to 10 days after inoculation of the main cats.

High amounts of viral RNA excreted by the respiratory and gastrointestinal tract are most likely responsible for transmission to sentinel animals.

Response to re-exposure (adult cats)

The re-exposed Group 1 cats (from study 2) were sampled for oral and nasal excretion for 7 days after exposure by viral isolation, and excretion was not detected in any cats at any time after the rechallenge (2).

Viral shedding produced by cats is relatively high and the speed of transmission is relatively rapid. Perhaps more importantly, cats develop significant neutralizing antibody titers and are resistant to reinfection.

The ease of transmission between domestic cats indicates an important public health need to study the possible chain of potential human-cat-human transmission.

Transmission between cats and humans

Reverse zoonotic transmission represents a relatively low risk to animal or public health in areas where human-to-human transmission remains high.

The prospects for transmission between animals are becoming increasingly important as a source of reintroduction into humans.

It is therefore important to better understand whether exposed animals could play a role in transmission.

From cat to man

The role of cats in zoonotic transmission remains an open question, but the relatively short duration of shedding and resistance to re-exposure suggests that the risk is very low, especially when cats are kept indoors.

From humans to cats (reverse zoonosis)

We carried out a study of 9 cats living in close contact with their owners (belonging to a veterinary community of 20 students) in which 2 students tested positive for COVID-19 and 11 others consecutively presented clinical signs such as fever, cough, anosmia, etc. between February 25 and March 18, 2020 compatible with a COVID-19 infection (15).

All owners were close to their pets in the same room (12-17m²), shared the same bed (100% of owners), and accepted face / hand licking (78%). No animals

tested positive for SARS-CoV-2 by RT-PCR and no antibodies to SARS-CoV-2 were detected in their blood using an immunoprecipitation test.

In addition, nasal and rectal swabs were taken for one week, starting on the day of blood collection (March 25) and all animals tested negative for the presence of SARS-CoV-2 by RT-PCR.

None of the animals included in this study had or were not infected with SARS-CoV-2, despite repeated daily intra-species contact on campus, and more importantly despite frequent and lasting contact with COVID-19 patients confined to small rooms.

These results support the evidence of a zero or very low COVID-19 infection rate in companion cats in a situation of repeated and close contact with infected humans. This suggests that the rate of transmission of SARS-CoV-2 between humans and pets under natural conditions is likely low. However, a more recent article (September // April) shows different results from a serological survey on companion animals carried out between May and June 2020 in two neighboring regions (to the east of France): Franche-Comté and Rhône-Alpes. (3)

There is a high prevalence of anti-SARS-CoV-2 antibodies in pets from COVID-19 + households. The study is conducted on domestic cats of laboratory-confirmed COVID-19 patients, and results in a high seroprevalence of anti-SARS-CoV-2 antibodies in 23.5% of cats (8/34) according to the criteria of positivity choose.

The HIV status was significantly higher in pets from COVID-19 + households compared to those with unknown owners (8 times higher). These results highlight the potential role of pets in the spread of the epidemic. Although it cannot prove with certainty that the 8 positive animals were infected with SARS-CoV-2, the much higher seroprevalence in animals from COVID-19 + households provides evidence that pets were infected with SARS-CoV-2.

The hypothesis that seems feasible when considering his two studies is that a reverse zoonosis is possible if infected owners expose their pets during an acute infection. This can be supported by the following 3rd study.

Serum samples were collected from cats in Wuhan, including 102 sampled after the COVID-19 outbreak and 39 before the outbreak (14). 14.7% of cat sera collected after the outbreak were positive for the receptor binding domain (RBD) of SARS-CoV-2 by indirect enzyme immunoassay (ELISA). Of the positive samples, 11 (11/15) had antibodies neutralizing SARS-CoV-2 with a titre ranging from 1/20 to 1/1080. Additionally, 3 of these cats belonging to COVID-19 patients had the highest neutralization titer. In contrast, sera collected from veterinary clinic cats and stray cats had much lower neutralizing activity, indicating that the high neutralization titers could be due to close contact between cats and COVID-19 patients. Although infection in stray cats has not been fully understood, it is reasonable to assume that these infections are likely due to contact with an environment polluted with SARS-CoV-2, or COVID-19 patients who have fed the cats.

These data show that SARS-CoV-2 infected a cat population in Wuhan during the outbreak. It is conceivable that the infected and seroconverted cats

identified in Wuhan, were in contact with patients whose viral load was higher than in the study above, or had more contact with infected cats, which would confirm the hypothesis made.

Conclusion

Unlike other pets, dogs do not transmit the virus to living things and are not susceptible to SARS-CoV 2 infection. There are very few cases of reverse zoonosis observed in this species to date.

Although further studies are needed due to the small number of cases and situations, we know that this virus does not cause symptoms in canines and that they are not playing a role in the pandemic.

However, they could help us detect infected cases of COVID-19. Indeed, being known for their overdeveloped sense of smell used in certain areas, a very recent study was carried out to determine whether dogs are able to detect the virus with samples of axillary sweat. An approach involving this study is currently being carried out in certain places such as the airport located in Helsinki, Finland. This finding could potentially prove to be more effective than current screening tests.

Although the sensitivity of cats to SARS-CoV-2 is now fairly clear, particularly thanks to the studies presented, its potential role as a reservoir for the virus remains questionable. In fact, the cat replicates the SARS-CoV-2 RNA and excretes the virus by air. But the short duration of excretion and its reactivity to re-exposure make it not very dangerous for humans.

In addition, it can be said from the various studies that cats transmit viruses to each other quite efficiently. In fact, the fear would be that cats would be contaminated by humans, and then contaminate each other exponentially to contaminate humans again.

Finally, a hypothesis can be put forward on the fact that humans transmit the virus to cats to a greater or lesser extent depending on the viral load.

As the studies are recent, with questionable aspects on several occasions, we cannot always obtain reliable answers or which can serve as a general conclusion. You must not forget to keep a critical mind in this kind of situation.

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SARS-COV-2 INFECTION IN LIVESTOCK

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Summary

In the context of the pandemic spread of coronavirus SARS-CoV-2 (Severe Acute Respiratory Syndrome-CoronaVirus-2) infection and the detections by RT-PCR as confirmation method, in February / March 2020, of numerous positive mink (from breeding of the captive wildlife) for SARS-CoV-2, which were in close contact with infected human patients, the following question was raised: what is the potential role of farm animals in the transmission and occurrence of this disease?

Keywords: SARS-CoV-2, livestock

During the month of December 2019, a new coronavirus (SARS-CoV-2) appears in Wuhan, China. This is responsible for the disease named Covid-19 in humans and has led to a global pandemic, calling into question the potential role of animals, in particular farm animals, in the transmission and onset of this disease. This virus which was unknown before 2019, is distinct from beta-coronaviruses, known to circulate in domestic animals (21). However, although the exact origin is not yet known, it probably has an animal origin (the role of a bat breed is assumed) with or without the intervention of an intermediate host, knowing that mink farms have been particularly affected by Covid-19 (10, 22). This article will outline the important points involving Covid-19 and livestock.

Origin

Coronaviruses represent a large family of enveloped positive single-stranded RNA viruses which belongs to the family *Coronaviridae*. Among them, there are many subfamilies, subgenera and genera. SARS-CoV (responsible for the Severe Acute Respiratory Syndrome – SARS - in 2002) and SARS-CoV-2 are classified in the genus *Betacoronavirus*. They belong to two genetically related groups. The SARS-CoV-2 belongs to the *Sarbecovirus* subgenera, which is different from the *Betacoronaviruses* from animals.

However, genetic analyzes have detected sequences close to this virus in *Rhinolophus* bats.

Coronaviruses in farm animals

Coronaviruses are classified in four genera: alpha (α CoV), beta (β CoV), gamma (γ CoV) and delta (δ CoV). They are responsible for infections in many poultry species (γ CoV, δ CoV) and also in mammals (α CoV, β CoV, γ CoV), including humans. The diseases caused in humans and domestic animals are different, but mainly affect the respiratory and digestive systems (20). The SARS-Cov2 virus is known to be part of the *Betacoronavirus* genus (subgenera *Sarbecovirus*) (7, 16, 28).

In cattle

Bovine coronaviruses (BCoV) are *Betacoronaviruses* (subgenera *Embecovirus*) and cause neonatal diarrhea in calves, winter dysentery in adults, and respiratory symptoms in all categories of age.

In horses

Equine coronaviruses (ECoV) are *Betacoronaviruses* (subgenera *Embecovirus*) and cause diarrhea. According to the observations of Pusterla et al. (14), horses which are used for work showed a higher rate of infection than other breeds, this could be explained by the stress induced by the working conditions.

In pigs

There are four porcine coronaviruses belonging to different genera of coronaviruses: *Alphacoronavirus* which contain porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus (PRCV). TGEV was associated with respiratory disorders; *Betacoronavirus* which contain hemagglutinating porcine encephalomyelitis virus (PHEV), responsible for vomiting and wasting disease; and *Deltacoronavirus* which contain coronavirus responsible for digestive disorders (PDCV) (13).

The other coronaviruses from domestic animals belong to other genera than *Betacoronavirus* (e.g., in cats, the feline coronavirus FCoV responsible for feline infectious peritonitis belongs to the genus *Alphacoronavirus*) and are even more genetically different from it (17, 23). In conclusion, the SARS-Cov2 is distinct (even if not direct genetic relationships) from the other coronaviruses which currently circulate in domestic animals (26).

Virus transmission to another species

SARS-CoV-2 belongs to viruses related to SARS-CoV whose reservoir is the bat. However, since the bats' places of life are far from human communities, the inter-species passage probably required an intermediate host, as was the palmate civet for SARS-CoV-1. In the case of SARS-CoV-2, the pangolin, a wild mammal consumed in particular in China and whose ecological niche covers that of bats, could have played this role, as suggested by the isolation of a strain of coronavirus from pangolin very close phylogenetically (92% homology). Furthermore, compared to SARS-CoV-1 and bat coronaviruses, SARS-CoV-2 exhibits a significant change

of the binding domain of the receptor located on the S protein and responsible for a gain in affinity for its ACE2 receiver. This binding domain is found almost identically (only one different amino acid) in a pangolin coronavirus, supporting the idea that the evolution of the virus in contact with the pangolin could have favored the passage to humans, possibly via the translocations of the binding domain (2). This cross-species transmission would have occurred in China, possibly at the Huanan market, since the majority of the first cases of COVID-19 were exposed there at the end of 2019 (27). Nevertheless, phylogenetic analysis of viruses isolated in China reveals that at least two different strains of SARS-CoV-2 had appeared several months before the first cases described.

ACE2 cell receiver

This virus requires, in order to infect cells, a receptor from the host cell membrane, to which it attaches, in order to initiate a fusion of the membranes. The SARS-CoV2's receptor has been identified as the angiotensin converting enzyme 2 (ACE2) receptor (5, 9). Experimentally, it has been identified as a receptor for other coronaviruses, including SARS-CoV which affect animals. This receptor is mainly present in the lungs, on the surface of cells of deep alveolar epithelia, but also on many other cells: enterocytes, renal cells, arterial and venous endothelia, arterial smooth muscles, epithelia of the small intestine (4).

In some of the animals, this receptor seems to be present, sometimes with differences compared to the ACE2 receptor from humans. In pigs, cats, ferrets, rats and bats, the receptor has amino acid sequences very close to the binding motif of human ACE2 (2). It should be noted that in pigs, four of the five amino acids in the binding domain are identical to those in humans. Cattle and poultry have different cell receptors than humans.

Animal species known to be susceptible to SARS-CoV-2

Different studies have been developed on several animal species to identify the grades of sensitivity.

Cattle

After intranasal inoculation of SARS-CoV-2, naive cattle were put together with infected cattle. Daily examinations noted that none of the animals showed signs of infection. However, viral RNA was identified and seroconversion occurred in two (of six) cattle, indicating viral replication. Intra-species transmission in cattle was absent. Cattle were therefore classified as having "low susceptibility", and not playing a role in pandemic COVID-19 (1, 15, 19).

Poultry (domestic species)

Some poultry species and embryos were experimentally inoculated with the virus. Daily clinical examinations and histopathological exams reveal no changes. Also, neither antibodies nor viral RNA were detected. It was concluded that turkeys

and chickens (as well as embryos) are not susceptible, and do not present any potential risk (3).

Pigs

After intranasal inoculation of sixteen pigs, two naive pigs were put together with infected pigs. Daily examinations and histopathological exams showed mild clinical signs: cough, nasal discharge, and mild depression in one case. Very low level of viral RNA was detected. Pigs were classified as "susceptible" although at low levels. Until today, no "natural" case has emerged (8, 18).

Rabbits

After experimental infection on rabbits, they were kept under observation for twenty-one day. None of the animals expressed clinical signs, but histopathologic exam indicated a mild to moderate increase the number of alveolar macrophages, as well as the presence of eosinophils in the lungs and nose. In some animals was seen enlarged tracheobronchial lymph nodes. Viral RNA was detected in all infected animals. The rabbits were therefore classified as "susceptible" species, with an asymptomatic infection (11).

Mustelids

Numerous studies have been done in ferrets and mink following SARS-CoV-2 infections in mink farms in the Netherlands (25). In these farms are noticed high mortality, with clinical signs such as: labored breathing, nasal discharge, anorexia, etc. Histological examination showed severe interstitial pneumonia, with necrosis of the epithelial cells of the bronchioles. Viral RNA has been detected. The mustelids were therefore classified as "sensitive" species (10, 12).

Sheep, goats, horses

No studies have yet been performed in these species. As no natural infection has been reported, they are probably "non-susceptible" (14, 24).

Potential role of food in the transmission of the virus

After several investigations, it was concluded that food can be contaminated, mostly by people, via infected droplets. The question is if the virus can be transmitted to human by meat.

Cooked food

To date, there is no sufficient data about inactivation of SARS-CoV-2 at high temperature, but is well known that other coronaviruses with zoonotic potential can be destroyed at high temperatures. Knowing this is easier to establish the value of temperature necessary for inactivation of the virus. So, cooking for 4 minutes at 63°C is supposed to be effective in inactivating coronaviruses (including SARS-CoV-2) in food.

Prepared or raw foods

Coronaviruses appear to be stable at low and negative temperatures, so refrigeration and freezing do not inactivate the virus. Thus, the virus could be transmitted in two ways:

1) Through digestive system: the ACE2 being present on the epithelial cells of the intestine, this could contribute to the infection of this one. However, SARS-CoV2 does not appear to have S proteins capable of binding to sialic acids, and therefore the virus is not protected from gastric acid secretion. The gastrointestinal symptoms would therefore be linked to a spread of the virus rather than a direct entry through the digestive tract.

2) Is by the respiratory tract: no risk of respiratory tract infection with coronaviruses after ingestion of a contaminated food has been observed.

In conclusion, to date, there is no evidence to suggest that the consumption of contaminated food can lead to an infection through the digestive tract, but the possibility of infection of the respiratory tract during chewing cannot however be completely excluded, in which case it is preferable to cook the meat. It should be remembered that good hygiene practices, if applied correctly when handling and preparing food, prevent contamination of food with the SARS CoV-2 virus.

Conclusions

Infection with coronaviruses is commonly in livestock, but the role of these animals in transmission and spread of SARS-CoV-2 is uncertain.

Infected animal products which come from these animals seems to represent a source of infection for humans.

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THE SURGICAL MANAGEMENT OF AN INGUINAL HERNIA IN A FEMALE DOG - A CASE REPORT

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Summary

An inguinal hernia, congenital or acquired, is a protrusion of an organ, fat or tissue through the inguinal ring. The hernia contents commonly includes: small intestine, omentum, spleen, urinary bladder, pregnant uterus or pyometra. The aim of this study is to report and discuss a case of an 8-year-old female dog referred to the Surgery Department of the Veterinary Teaching Hospital of Iași with a history of caudal abdominal swelling, progressively growing in the last four weeks. The clinical examination had shown a soft, painful, irreducible mass in the left inguinal region, and the diagnosis of inguinal hernia was later confirmed through ultrasonography. Surgical exploration revealed herniation of both uterine horns with their broad ligaments, the uterine body, omentum and small intestine. During the surgery, herniorrhaphy and a routine ovariohysterectomy were performed. No postoperative complications were recorded and the patient recovered uneventfully. The particular element in this case is represented by the movement of the uterus into the hernia sac, through the inguinal canal, even though the female was unpregnant and other potential risk factors were also not fulfilled.

Keywords: inguinal hernia, uterus, herniorrhaphy, dog

Abdominal hernia is defined as the protrusion of abdominal contents through a congenital or acquired defect named hernia ring. A systematic classification (Fig. 1) of the different types of hernia is invaluable, because the correct identification of the hernia will allow us to select the proper protocol.

Inguinal hernias are protrusions of organs or tissues into the inguinal canal, through the inguinal ring. This disorder may arise from a congenital abnormality of the inguinal ring, or may be caused by trauma (1). The congenital inguinal hernia in dogs is much more frequent in males than in females, possibly because of delayed inguinal ring narrowing due to a late testicular descent (4, 10). The most commonly predisposed breeds are Pekingese, Besenji, Poodle, Basset hound, Chihuahua, Cocker spaniel, Pomeranian, Maltese and West Highland Terrier (7). The acquired ones are more common in bitches, most often involving the middle aged intact bitches. This aspect is confirmed by Waters (16) in his retrospective study that conclude the fact that 73% of acquired hernia concerns females, and that fat and omentum were the most common hernial contents. The hernia contents commonly includes: small intestine, omentum, spleen, colon, urinary bladder, pregnant uterus or pyometra (12, 16).

A successful surgical repair of inguinal hernias depends on knowledge of regional anatomy and the appropriate surgical technique. Inguinal canal is physiologically open in carnivores. The round ligament (*Ligamentum teres uteri*) passes through the inguinal rings, and his fibres recline in *procesus vaginalis* in bitches (9). It is considered that this ligament may direct uterus into the vaginal sac at pregnancy and therefore, hernia may occur (8). If the uterus protrude in the hernia sac it will limit the movement of the small intestine in the sac; such hernia is likely to be large and a large hernia is usually associated with less risk for incarceration (15). In both sexes, the external pudendal vessel and the genital branch of the genitofemoral nerve, artery and vein pass through the caudomedial aspect of the canal. In male dogs the spermatic cord and the cremaster muscle also pass through the external inguinal ring (4). This anatomical features are very important since occasionally we have to enlarge the herniar ring for the hernia content to be replaced easily through the defect. The cut will always be done in the cranio-lateral side of the inguinal ring, because of the vital structures that are passing through the caudomedial aspect (Fig. 7).

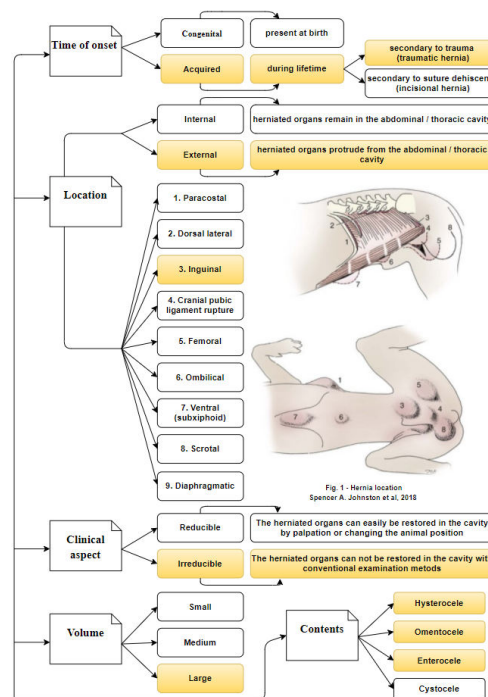


Fig. 1. Hernia classification – The coloured rectangles contain the description of the hernia presented in this article

Besides the anatomy of the inguinal canal (shorter and larger in females than in males), hormonal variation, increased intraabdominal pressure in pregnancy or obesity, and atrophy of the abdominal wall due to old age, are other potential risk factors for this disease (11, 12). Sex hormones, particularly estrogen, have a role in the inguinal hernia pathogenesis, because the majority of them appear during estrus or pregnancy and have not been reported in spayed females. Estrogen could modify the character and strength of the connective tissue, ligaments, weakening and enlarging the inguinal rings (12, 15). Nutritional or metabolic diseases, such as hyperadrenocorticism and diabetes mellitus, influence the strength of the abdominal wall. Obesity increases the abdominal pressure forcing the abdominal fat movement through the inguinal canals (7). Because of the potential heritability in some breeds, future breeding of female dogs with inguinal hernia is improper and ovariohysterectomy is recommended (13).

Materials and methods

The study was conducted on an 8-year-old female dog referred to the Surgery Department of the Veterinary Teaching Hospital of Iași with a history of caudal abdominal swelling, progressively growing in the last four weeks. The owner indicated that the discomfort and pain started to appear in the last few days, and until now the animal did not seem to have any discomfort. Reproductive parameters such as estrus cycle intervals, duration of heat, sexual status, or past pregnancies were not known. The owner further noted that food and water intake were normal and no constipation, vomiting or urinary disturbance were observed. Vomiting is predictive for nonviable small intestines and the herniorrhaphy would have become an emergency procedure.

At a primary examination the bitch appeared healthy, with normal body temperature (38,9°C), respiratory rate (30 bpm), heart rate (127 bpm) and good general body condition. No abnormalities were reported on thoracic auscultation.

In the differential diagnosis, a distinction between inguinal herniation, mammary gland tumor, mastitis, local abscess, lymph node hypertrophy, hematoma and granuloma had to be made (11, 13).

The physical examination had shown a soft, painful, irreducible mass in the left inguinal region. The swelling could not be manually reduced by palpation or by changing the animal position, although several attempts were made. However, pain was determined in palpation, therefore ultrasonography of both the mass and abdominal cavity was performed. No modified structures such as pyometra or pregnancy were found in the ultrasonographic examination of the abdominal region. Mass ultrasonography revealed hypoechoic circular areas indicating intestinal loops and a tubular structure presumed to be the uterus as neither pulsation or peristaltic activity could be identified.

Biochemistry, haematology and urinalysis results were unremarkable.

After the clinical and ultrasonographic examination of the mass, a presumptive diagnosis of inguinal hernia was rendered and a surgical repair was decided.

Surgical management consists of hernia sac identification, assessment of the hernia contents viability, surgical resection of nonviable tissue if needed, abdominal reduction and neutering if needed.

After fasting for 12 hours, the patient was prepared for surgery.

The female was premedicated with atropine (0.04 mg/kg) subcutaneously, and ten minutes later anaesthesia was induced by xylazine (2 mg/kg) and ketamine (15 mg/kg) intramuscularly.

The patient was placed in dorsal recumbency and the inguinal side was aseptically prepared for surgery (Fig. 2 - 3).



Fig. 2. Clinical aspect – inguinal hernia



Fig. 3. Aseptic field preparation

The surgical approach includes a skin incision, made directly over the swelling. The incision was made using a scissor, in order not to damage the herniar content, and then extended cranially and caudally (Fig. 4). The subcutaneous tissue is dissected from the abdominal fascia, and the hernia sac is exposed (Fig. 5).

Results and discussions

The hernia sac is exposed and, through it's transparency, it can be seen the small intestine and the uterus (Fig. 6).

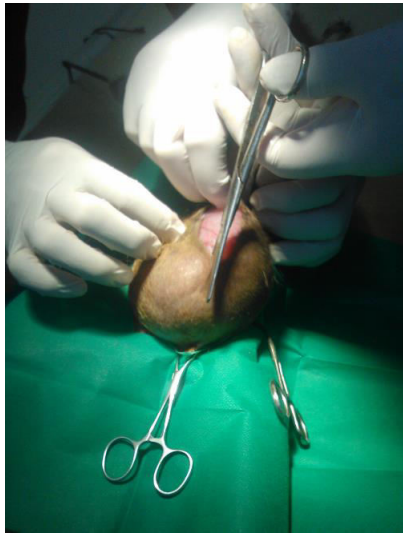


Fig. 4. Skin incision



Fig. 5. Subcutaneous dissection

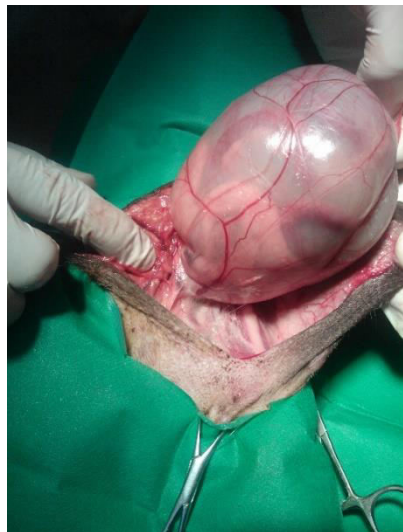


Fig. 6. Hernia sac

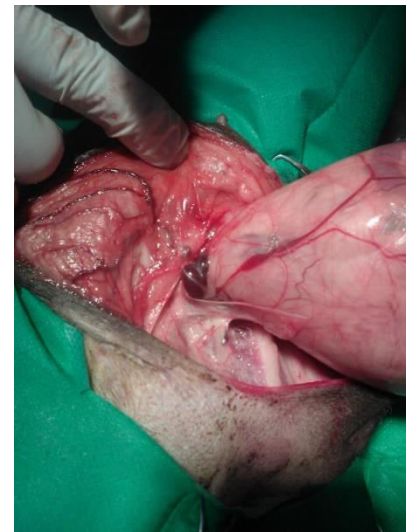


Fig. 7. Pudendal vein

Due to the large size of the hernia, the inguinal ring was extended cranially (Fig. 8) with a blunt scissor to allow the reduction of the hernia content into the abdominal cavity. Because the hernia was not easily reducible, the sac was opened.

An incision was made over the hernial sac and hernia contents integrity was examined (Fig. 9). No lesions, abnormal temperature or color change were noticed on the uterine horns with their broad ligaments, the uterine body, omentum and small intestine, therefore it was safe to gently reduce them back. Before closing the defect, an ovariohysterectomy was performed through the enlarged inguinal ring. In all previous cases of inguinal hysterocele, after the closure of the inguinal ring, the ovariohysterectomy was made via midline incision. In this case, the ovariohysterectomy was made through the same incision as the hernia repair, because we already had to extend the incision cranially in order to reduce the hernial contents.

Excessive hernial sac was cut and margins apposed in a simple continuous pattern using a 2-0 monofilament prolonged absorbable material. When closing the abdominal defect, care must be taken not to damage the vital structures that pass through the inguinal ring; therefore the caudomedial aspect of the ring, that contains the external pudendal artery and vein and genitofemoral nerve, was not sutured. (Fig. 10).

Excessive skin tissue was also trimmed off for a correct apposal, and the dead space was reduced, by closing the subcutis in a continuous suture pattern with 2-0 absorbable suture material, before skin closure (Fig. 11).

The patient was discharged home in the same day, after the surgical procedure. Post operative antibiotic and anti-inflammatory therapy with Amoxicillin (15 mg/kg) IM and Meloxicam (0.2 mg/kg) SC was administered immediately after surgery. Oral antibiotics and antiinflammatory were administered for 5 days postoperative.

Postoperative considerations included placing an Elizabethan collar until removing skin sutures and limited activity during recovery. A pressure bandaging was also advised.

Usually, the clinical symptoms exhibited by the animal reflect the size, the content and the severity of the hernia (5). It ranges from a painless inguinal mass to signs related to incarcerated or nonviable small intestine. Ultrasonography and radiography are invaluable diagnostic tools that can be used in assessing the organs involved in the hernia. This will help planning for surgery and establishing the prognostic (15).

The presence of the uterus inside the hernia sac may remain unnoticed unless there is a dilatation due to pregnancy, an uterine affection, or for instance when pyometra occurs (2). Left-sided occurrence is common. This aspect is confirmed by Itoh in his retrospective study that conclude the fact that fourteen of fifteen uterine herniation (93%) were located left side (3).

The surgical repair of this condition involves preoperative, operative and postoperative considerations. Diet restrictions, stabilisation of the patient, antibiotherapy and analgesy are very important.

The female dog recovered uneventfully with no postoperative complications. The owner reported that the patient was clinically normal without any recurrence of the swelling three months later.

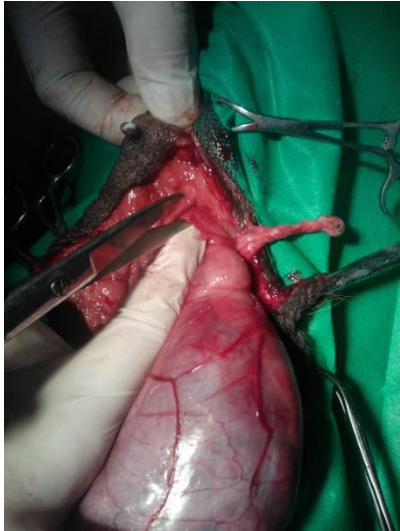


Fig. 8. Inguinal ring enlargement



Fig. 9. Hernia contents

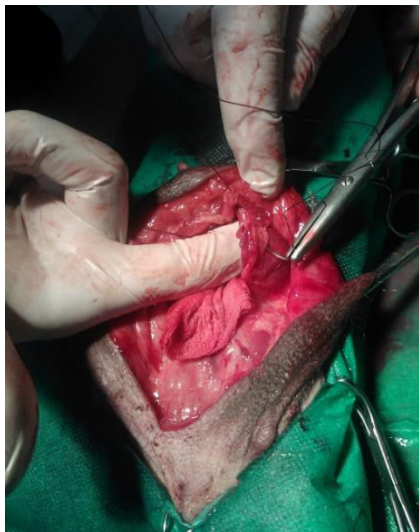


Fig. 10. Inguinal ring closure



Fig. 11. Excessive skin removal

Common postoperative complications in inguinal hernia surgery repair include incisional infection followed by wound dehiscence, seroma, hematoma, edema, excessive post operative swelling, hernia recurrence, peritonitis, sepsis and

death (15). The routine usage of surgical drains in the management of likehood postoperative seroma formation in dogs is not strongly recomanded. In a retrospective study although surgical drains were used only in two of 30 nontraumatic inguinal hernias, none seroma was reported (16).

Conclusions

In conclusion, even if the uterine herniation is usually diagnosed during estrus stage or pregnancy, when estrogens interfere with the connective tissue function, inguinal hysterocele must be considered even though the female is not pregnant, obese or has pyometra. In bitches the round ligament (*Ligamentum teres uteri*) passes through the inguinal ring, and its fibres recline in procesus vaginalis. The female dog is the only animal that has procesus vaginalis (4) and this aspect will always predispose her to uterus herniation.

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THE EVALUATION OF SPECIFIC CLIMATIC FACTORS IN THE MOUNTAINOUS REGION OF DORNA DEPRESSION ON RAW COW MILK FAT AND PROTEIN CONTENT

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Summary

Geoclimatic factors have a heavy influence upon animal productions, their welfare and health through their action on animals and the biodiversity of the feed source. All these factors also have a major impact on the quality and milk yield of cows raised in different geographic regions. This study has the aim of evaluating the influence of seasonal climatic factors (temperature, atmospheric pressure, humidity, precipitations) on the compositional indices of commercial interest (fat and protein) of raw milk obtained from cows raised in the mountainous region of Dorna. The study was conducted over a period of time starting from November 2019 to September 2020, using milk obtained from a private commercial company situated in the south-vest of the Bucovina region in Suceava county. The research was based on the collaboration with two weather stations which provided information regarding the seasonal dynamic of temperature, humidity, precipitations and atmospheric pressure. The influence of the climatic factors was monitored through the testing of compositional fat, protein and non-fat dry matter content of raw milk on samples (n=30). The obtained data was statistically analyzed using current methods of biostatistics (ANOVA Software and Tukey-Kramer Multiple Comparisons Test). The analysis of the seasonal evolution of compositional parameters of raw milk revealed the following: the samples from private households recorded a drop in fat content during summer (3.58 %) and a rise during autumn (3.84%), whereas for micro farms the drop was recorded in winter (3.71%) and higher values in autumn (3.88%); as for the protein content for samples that came from private households the lower values were noticed during autumn (3.75%) and higher ones during summer (3.38%), whereas for micro farms the lower values were recorded during autumn (3.17%) and the higher values in winter (3.40%); and lastly for the non-fat dry matter content registered in samples from private households recorded a drop in winter (6.04%) and a raise in autumn (6.32%), respectively for micro farms the drop was registered in the spring (6.18%) and the values raised again in winter (6.33%). The effects of the dynamic of the main climatic factors on the compositional parameters of raw milk revealed that temperature oscillations directly influence the fat content and the variations of humidity and rainfall mainly influence the protein and non-fat dry matter contents.

Keywords: cows, raw milk, fat, protein, Dorna Depression.

Milk and dairy products have a very important role in the human nutrition because they provide a rich source of organic compounds (such as protein, lipids, carbohydrates, vitamins) and high quality minerals. Nowadays there is a large variety of dairy products with organoleptic characteristics, physico-chemical and microbiologic properties that are adapted to the preferential needs of the consumers.

Based on these recitals there we differ conventional dairy products that have been obtained using standard recipes and technologies, traditional dairy products that come from a specific geographical area and are made using special recipes and ecological or organic dairy products that are made out of materials that came from ecological agriculture (15). Climatic factors exert a major impact on the expression of the production potential of livestock thus influencing the quantity and quality of the resulted milk and thus, dairy products (16). It is a known fact that milk yield is influenced by the climate-animal relationship and especially by the quality of the environment, all of these factors are reflected on the welfare of dairy cattle (17). The influence of the climatic factors are wielded directly on the animal organism, as well as indirectly through the biodiversity of the pastureland and the feed, thus producing important individual and seasonal variations on the quantity and quality of the milk and dairy products (13). Out of the climatic factors that can produce major effects on the milk yield in cows are temperature, humidity, atmospheric pressure, rainfall, light and altitude. The ambient temperature is the main factor that has great influence on yielding in general and on the milk yield especially (11). The area of thermal comfort for dairy cattle is situated between 9-16°C; if the ambient temperature is situated within this range then the animal organism won't resort to additional physio-chemical processes to ensure thermoregulation (14). The resistance of cattle to temperature oscillation is heavily influenced by certain intrinsic factors like species, breed, the colouring and the grossness of the hair (3). The air humidity is strongly related to the temperature variations, so the optimal rate is situated between 65-75%. Low humidity associated with high temperatures intensify the thermoregulation process, whereas high humidity regarding temperature values do not have the same effect on thermoregulation (5, 6). Light intensifies the metabolic processes, having a positive effect on the milk yield. Humidity, especially excessive rainfall, causes a drop in the milk yield through inadequate feeding of the cattle and the stressful effects they have on the organism. Constant attitude can influence the animal organism through the enhancement of the oxidative processes and by default lacto genesis.

Materials and methods

The main interest of this study is the evaluation of the physico-chemical, hygienic and sanitary parameters of raw milk through the following fat (%), protein (%), non-fat dry matter (NFDM-%), density (G/cm³), freezing point (°C), pH, somatic cell count (103Cel/mL) and bacteria count (103UFC/mL). The tests have been made on samples of milk produced in mountainous region of Dorna and they were processed in a private processing unit in the region. The milk samples came from indigenous cow breeds from private households and micro farms type A. Small-scale producers still represent the main source of milk production in the area, each of them owning 2-10 cattle. In these private households dairy cows are raised in a traditional way and the milking is done manually. Together with the private household the study was also conducted on 8 local micro farms that have 10 to 30 cattle each. The cattle

from the micro farms are raised in good hygienic facilities and the milking is done using automatic systems. Each of these micro farms have their own cooling tanks to store the milk production. The cattle breed that is mainly raised in this area is Transylvanian Pinzgau and a couple of Black Dorna Pinzgau, whereas Romanian Spotted and Maramuresan Brown breeds are a rarity. Regarding the Pinzgau breed, it is mandatory to specify the fact that this group includes cattle that have adapted to the mountainous living conditions, thus they acquired a strong resistance to disease and the harsh environmental conditions. Another important thing to mention is the fact that in the region of Dorna the cattle benefit from seasonal natural grazing during the spring and summer seasons, and during the cold seasons the feeding is mainly done in the shelters and consists of fibrous plants (hay and grass). The study initially focused on finding suppliers that are in keeping with the requirements of the current legislation and the processing unit. The study thus included two main sources of the raw milk namely private households- small scale producers (n=60) and microfarms (n=8). The milk was collected from the suppliers via three routes, one of them was done using a truck with a standard milk tank, and the other two using horse-drawn carts. The two sources produce 22.000-35.000 L of milk on a daily basis, the quantity records seasonal variations. The study was conducted over a 12 month period (from November 2019- October 2020), in which time milk samples coming from both types of sources were categorized into two groups: samples A included the milk that came from private households and B samples that represented the milk that came from micro farms. From each samples, probes were collected and analyzed as representative milk samples (n=30). The final count of the total of analyzed samples reached 720 so 360 samples from each source.

The physico-chemical parameters of raw milk were tested by using the semi-automatic analyser Ekomilk M (Milkana kam 98-2A). The biochemical indices that have been evaluated are the seasonal evolution of fat content (%), protein (%) and the non-fat dry matter (NFDM-%) of raw milk. The analysis of the seasonal dynamic included the evaluation of the main climatic factors (temperature, atmospheric pressure, humidity and rainfall) on the compositional indices of raw milk. This was possible through a collaboration with two weather stations from the Dorna area that have provided data from 1500 m altitude regarding the seasonal dynamic of temperature, atmospheric pressure humidity and rainfall. The data resulted from the testing of the milk samples was initially processed and orderly categorised based on the investigated parameters and then distributed on private households and micro farms. The values of fat content, protein and NFDM of the milk samples from both sources were then inserted in statistical calculation used to determine the seasonal influence of climatic factors on the quality of the milk from mountain raised cattle. The statistical analysis was done using both the ANOVA system and the Tukey Test in order to determine the level of statistical significance of the differences between compositional parameter values of raw milk, as well as for the variables between private households- micro farms and seasonal variations of compositional parameters of milk.

Results and discussions

The analysis of the impact of the main climatic factors on the composition and quality of the mountain raised cattle milk is extremely relevant for specific character of this area. Their main feature is the alternation of sunny days and humid rainy days, that is important seasonal variation of temperature. It is well known that the cumulative actions of pedoclimatic conditions in mountainous regions ensure the cattle good health and by default a high quality milk and dairy products. This study is focused on analyzing the impact of the main climatic factors (temperature, humidity, atmospheric pressure and rainfall) on compositional indices of raw milk.

The analysis of the seasonal variation of the fat content of raw milk revealed lower average values in summer (3.58%) and higher average values in autumn (3.84%) for private households. In micro farms the recorded average values were lower during winter (3.71%) and higher in autumn (3.88%) (Table 1). The statistical differences were insignificant ($P>0.05$) for all four seasons (Table 2). The same evolution is seen in the charts of the diagrams one and two that show lower values being recorded in the summer and the winter and higher values in autumn, without significant statistical differences among seasons. Through the seasonal comparative analysis of the milk fat content for both sources it was determined that samples from private households revealed that the minimum values were 3.62% and the maximum 3.75%, whereas a minimum of 3.64% and maximum of 3.75% in the case of micro farms (Table 1).

The seasonal evolution of the protein content for the milk that came from private household was higher during autumn (3.75%) and lower during summer (3.38%), whilst for micro farms the values were lower in autumn (3.17%) and higher in the winter (3.40%) (Table 1), without significant statistical differences ($P>0.05$) between the four seasons (Table 2). The same distribution can be seen in the charts of Figure 1 and 2, that reveal the protein content rise during autumn and drop during summer and winter, this without recording significant statistical differences between seasons. Regarding the seasonal evolution of milk's protein content of the raw milk from the two different sources it was established that the samples harvested from the private households had a minimum of 3.24% and a maximum of 3.34%, whereas the samples from the micro farms were 3.20% and respectively 3.69% (Table 1).

The seasonal distribution of the non-fat dry matter content of raw milk samples from private households revealed lower average values during winter (6.04%) and higher values during autumn, whereas the lower values recorded in micro farms were during spring (6.18%) and the higher values in winter (6.33%) (Table 1). There were no relevant statistical differences ($P>0.05$) recorded between seasons (Table 2).

Table 1

The distribution of the statistical values of the main compositional parameters of raw milk samples collected from private households and micro farms from the Dorna region

Season	Parameter	PRIVATE HOUSEHOLDS (n=360)			MICRO FARMS (n=360)		
		Fat (%)	Protein (%)	NFDM (%)	Fat (%)	Protein (%)	NFDM (%)
WINTER	Minimum	3.620	3.240	5.890	3.640	3.200	6.240
	Median	3.700	3.330	6.020	3.750	3.320	6.280
	Maximum	3.750	3.340	6.230	3.750	3.690	6.480
	Average	3.690	3.303	6.047	3.713	3.403	6.333
	STDEV	0.06557	0.05508	0.1716	0.06351	0.2554	0.1286
SPRING	Minimum	3.690	3.240	6.190	3.700	3.240	6.110
	Median	3.700	3.350	6.250	3.720	3.260	6.170
	Maximum	3.700	3.390	6.260	3.750	3.640	6.280
	Average	3.697	3.327	6.233	3.723	3.380	6.187
	STDEV	0.005774	0.07767	0.03786	0.02517	0.2254	0.08622
SUMMER	Minimum	3.480	3.250	5.990	3.640	3.220	6.100
	Median	3.630	3.630	6.110	3.840	3.240	6.260
	Maximum	3.640	3.640	6.360	3.850	3.390	6.340
	Average	3.583	3.380	6.153	3.777	3.283	6.233
	STDEV	0.08963	0.2252	0.1888	0.1185	0.09292	0.1222
AUTUMN	Minimum	3.720	3.040	6.260	3.750	3.140	6.100
	Median	3.880	3.210	6.350	3.930	3.180	6.390
	Maximum	3.920	3.250	6.370	3.980	3.200	6.490
	Average	3.840	3.167	6.327	3.887	3.173	6.327
	STDEV	0.1058	0.1115	0.05859	0.1210	0.03055	0.2026

The charts illustrated in the first and second diagrams show the evolution of the non-fat dry matter content of raw milk namely the average drop recorded in samples collected from private households during winter and the rise of the average values in spring. As for micro farms, there is a recorded drop in the non-fat dry matter values during spring and a rise noted during winter. There are no notable statistical

differences between seasons. The seasonal evolution of this parameter is also reflected within the minimum and maximum values obtained from the sample analysis from the two different sources, so for private households the values were 5.89% respectively 6.23%, as for micro farms the values were 6.24% and 6.48% (Table 1).

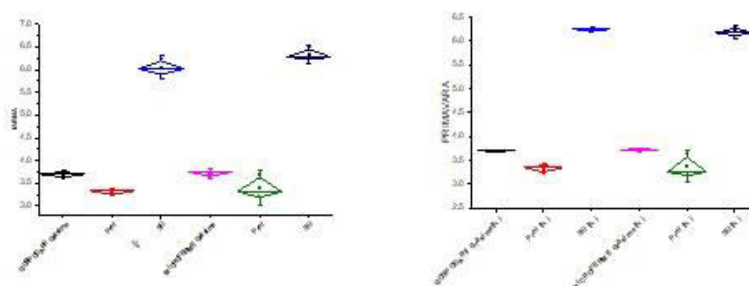


Fig. 1. The distribution raw milk parameters in the winter and spring season

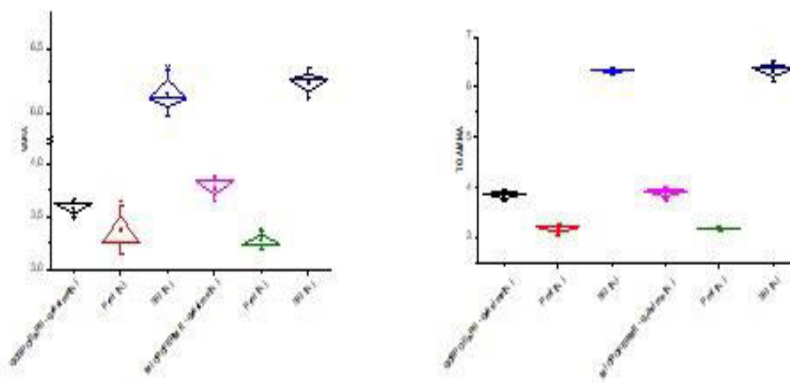


Fig. 2. The distribution of raw milk parameters in the summer and autumn season

Table 2

The statistical results of the main compositional parameters of raw milk after the Tukey-Kramer Multiple Comparisons Test

Season	Parametre	PRIVATE HOUSEHOLDS (n=360)			MICRO FARMS (n=360)		
		Fat (%)	Protein (%)	NFDM (%)	Fat (%)	Protein (%)	NFDM (%)
Winter (I)	Average	3.690	3.303	6.047	3.713	3.403	6.333
Spring (P)	Average	3.697	3.327	6.233	3.723	3.380	6.187
Summer (V)	Average	3.583	3.380	6.153	3.777	3.283	6.233
Autumn (T)	Average	3.840	3.167	6.327	3.887	3.173	6.327
Fat content comparison		<i>Mean difference</i>		<i>q</i>	<i>P Value</i>		
	I vs P	-0.008500		0.1678	ns P>0.05		
	I vs V	0.02150		0.4245	ns P>0.05		
	I vs T	-0.1620		3.198	ns P>0.05		
	P vs V	0.03000		0.5923	ns P>0.05		
	P vs T	-0.1535		3.030	ns P>0.05		
	V vs T	-0.1835		3.623	ns P>0.05		
Protein content comparison		<i>Mean difference</i>		<i>q</i>	<i>P Value</i>		
	I vs P	-0.0005000		0.01341	ns P>0.05		
	I vs V	0.02150		0.5765	ns P>0.05		
	I vs T	0.1830		4.907	ns P>0.05		
	P vs V	0.02200		0.5899	ns P>0.05		
	P vs T	0.1835		4.920	ns P>0.05		
	V vs T	0.1615		4.330	ns P>0.05		
NFDM content comparison		<i>Mean difference</i>		<i>q</i>	<i>P Value</i>		
	I vs P	-0.02000		0.2662	ns P>0.05		
	I vs V	-0.003000		0.03993	ns P>0.05		
	I vs T	-0.1370		1.824	ns P>0.05		
	P vs V	0.01700		0.2263	ns P>0.05		
	P vs T	-0.1170		1.557	ns P>0.05		
	V vs T	-0.1340		1.784	ns P>0.05		

The processing of the data provided by the two weather stations provided information regarding the monthly and seasonal evolution of temperature, atmospheric pressure, humidity and rainfall values at an altitude of over 1500 m in the Dorna area. The dynamic of these climatic factors are recorded in Table 3, so the recorded variables in 2020 are the following: the recorded temperature was situated

between -9.8°C (in January) to +16.6°C (in August), with a multiannual average of +4.10°C; the humidity levels recorded were between 75% (in April) and 93% (in January), with a multiannual average of 82.62%; the rainfall values were between 12.0 L/m² (in April) and 202.6 L/m² (in May), with a recorded multiannual average of 69.25 L/m²; the atmospheric pressure levels were between 782.8 mb (in January) and 914.0 mb (August), with a multiannual average of 852.68 mb. It is important to specify that the oscillations of the recorded values of the climatic factors are within normal range for the Carpathian mountainous region (17). The analysis of the evolution of climatic factors revealed that the moderate temperatures recorded in the autumn had a positive effect on the fat content of milk samples from both sources, and none on the protein and non-fat dry matter contents.

Table 3

Average annual values recorded by the local weather stations in 2020

Month	Temperature (°C)		Humidity (%)		Precipitations (L/m ²)		Air pressure (mb)	
	I	II	I	II	I	II	I	II
January	-5.3	-9.8	93	91	34.3	82.2	901.4	782.8
February	-3.2	-5.5	86	70	12.0	77.3	912.9	794.3
March	1.3	-4.5	76	80	43.5	36.2	909.1	792.3
April	5.8	0.9	75	86	33.0	33.4	909.6	794.4
May	10.4	3.4	86	94	187.4	202.6	906.2	793.1
June	16.2	10.4	82	85	65.3	140.1	913.8	802.4
July	14.8	8.9	81	83	74.7	92.5	909.6	798.2
August	16.6	11.2	81	82	56.4	90.1	914.0	802.9
September	11.3	6.1	79	79	45.8	89.1	913.7	800.7
October	6.5	5.3	83	61	24.5	46.4	912.9	799.3
November	5.1	0.5	89	90	34.9	80.1	907.9	793.5
December	-1.4	-4.6	91	80	48.5	31.8	908.3	791.1
Mean	6.5	1.7	83.5	81.75	55.025	83.48	909.95	795.41
The yearly average	4.10		82.62		69.25		852.68	

Weather stations: I - Poiana Stampei; II - Călimani

It has to be noted the fact that other researchers in the field came to similar conclusions in their conducted studies regarding the influence of temperature on the main compositional parameters of milk (15). On the other hand it is important to mention the fact that humidity had a notable influence on fat and protein content and on the non-fat dry matter as well correlating with the milk's high protein levels during

summer, autumn and winter. The dynamic of the atmosphere pressure had a heavy influence on the fat content during autumn, winter and spring alike. Regarding this matter the statistical correlations that Someșan et al. (17) established in 2015 were recorded within the protein and NFDM values are relevant.

As for the influence of the dynamic of rainfall it is to be noted the fact that it didn't have a significant influence on the fat content, instead other researchers recorded a relevant statistical influence between the values of the protein content in autumn and for the unfat dry substance content in summer (17). According to our data the dynamic of atmospheric pressure did not influence the content of the three main components of raw milk. In this context it is important to specify that the possible influence of the atmospheric pressure on the compositional parameters of raw milk are less known, these influences only having a visible effect on the physical indices of milk (10, 17). The resulted conclusions of this study are similar to those of other conducted studies in the field, and they that the evolution of the fat, protein and unfat dry substance contents and the whole composition of milk implicitly, varies depending on the environmental conditions, temperature having the most impact, followed by humidity and atmospheric pressure (12, 15).

It is well known that different climatic factors have major effects on the process of lactation in cattle such as temperature, atmospheric pressure, light, rainfall and altitude. The most frequently monitored parameters are in fact temperature and humidity because they are considered to have the most significant impact on lactating cattle, respectively on the composition and the quality of the milk (1, 9). In this regard, the recent conducted studies centered around evaluating the effects of the environment on the composition of the milk suggested that cows with a big milk production are notably more susceptible to thermal stress than cattle with lower milk productions (2). The effect of temperature on the milk production was quantified through the study of climatic factors (temperature, humidity, the speed of the wind and light), the results suggesting a drop in milk production as a result of thermic stress (7, 8). In order to manufacture traditional dairy products an important role lies with the mountainous geo-climatic factors, that can easily influence the welfare and health of the animals, especially on the compositional and microbiological characteristics of raw milk (4, 13, 17).

Conclusions

The analysis of the seasonal evolution of the average values of the compositional parameters of raw milk for private household showed a drop of fat content in summertime (3.58%) and a rise in the autumn (3.84%), whereas for micro farms the drop was recorded during winter (3.71%) and the rise during autumn (3.88%).

The protein content recorded in private households dropped in summer (3.38%) and rose again during autumn (3.75%), and as for micro farms the protein content was lower in autumn (3.17%) and higher during winter (3.40%).

The non-fat dry matter content of samples that came from private households was lower during winter (6.04%) and higher during springtime (6.32%); the recorded results from samples collected from micro farms were lower during spring (6.18%) and higher in winter.

The effects of the dynamic of the main climatic factors on the compositional parameters of raw milk revealed that temperature oscillations directly influence the fat content and the variations of humidity and rainfall mainly influence the protein and non-fat dry matter contents.

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ECHOCARDIOGRAPHIC AND BLOOD BIOCHEMICAL INVESTIGATIONS IN ENDOCARDIOSIS IN DOGS

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Summary

Endocardial diseases, especially degenerative process, are common in dogs, especially in the elderly, Investigations were performed on a number of 14 dogs of different breeds and aged between 6 and 17 years who showed symptoms of a cardiovascular nature. In addition to echocardiographic investigations module B, B + M, color Doppler, PW-Doppler, a series of blood biochemical parameters specific to the cardiovascular system, creatin phosphokinase-CPK, glutamat oxal acetat transaminasis-TGO, lactate dehydrogenase-LDH were also investigated. Determinations were also made regarding a cardiac marker, troponin T, being an early indicator of cardiac dysfunction.

Keywords: dog, echocardiography, CPK, TGO, LDH, troponin T

Endocardiosis is suspected to be of genetic origin, representing a myeloid degeneration of the valves (mitral, tricuspid, sigmoid) and of the subvalvular device.

This morbid entity leads to deformations of the free margin of the valves, which subsequently determines their thickening and fibrosis, being responsible for the malfunction of the valvular apparatus and the consecutive presence of a regurgitation (6).

Mitral endocardiosis is the most common disease in canine cardiology, being more common in older dogs. About 60% of animals with endocardial insufficiency have lesions in the mitral valve, with a tricuspid involvement of 35% (4).

Specialized investigations (echocardiographic) corroborated with the values of certain blood biochemical parameters with cardiac specificity regarding the screening of cardiac disorders in dogs, allow the diagnosis of functional (frequency,

rhythm) and morphological changes at the level of atria, ventricles, interventricular septum, parietal and valvular endocardium (1, 2, 3).

In this study we proposed the monitoring and screening of degenerative diseases of the endocardium in dogs by corroborating the echocardiographic aspects (mode B, Doppler color) with the values of blood biochemical parameters with myocardial specificity (creatinfosfokinase - CPK, glutamate-oxalacetate transaminase – GOT, TGO, lactate dehydrogenase - LDH, troponin T - TnT) (5).

Materials and methods

The study was carried out on a number of 14 adult dogs, of different breeds, with the weight between 6-52 kg and the age between 6-17 years, which from a clinical point of view had cardiac symptoms expressed by dyspnoea, cough, accelerated breathing, effortless intolerance. The investigations were carried out at the Medical Pathology discipline within the FMV Timisoara, in private veterinary medical offices in Timisoara, Italy and the blood biochemical investigations in the laboratory for medical analysis Bioclinica. The determination of blood biochemical parameters (CPK, TGO, LDN, TnT) was performed from blood collected from the antebrachial cephalic vein in vacuum tubes, provided with activating gel. Three types of ultrasound were used for the echocardiography: two stationary and one portable (X Vision MyLab 70 Vet, GE Logyq Er7, GE VIVID) and a mobile one, Mindray 2200 Vet), using micro-linear cardiac probes, with a frequency between 3.5-8 MHz and a microconvex probe with a frequency between 5-8 MHz. Statistical processing was performed using the IBM SPSS software 22 calculating the mean and dispersion indices for each analyzed parameter, and testing the significance of the differences between the mean values was performed using the ANOVA and Turkey test.

Results and discussions

Echocardiography, as a paraclinical method of endocardiosis in dogs investigating, shows four evolutionary stages: type 1 and 2, in which the lesions consist of small nodular thickening, more or less extended at the level of the cuspids, without involving the tendinous chordae; type 3, where there are lesions in the cuspids and type 4, characterized by lesions extended to the tendinous chordae, frequently, being able to determine their rupture.

In the case of an endocardial disease, the cord undergoes changes in reducing the elasticity of the parietal and valvular endocardium, causing increased tension on the papillary muscles and tendinous chordae. In particular, the increase of the intra-atrial pressure, in the first phase, determines a dilatative morphostructural change, concretized aspect, in particular, at the level of the left atrium (Fig. 1).

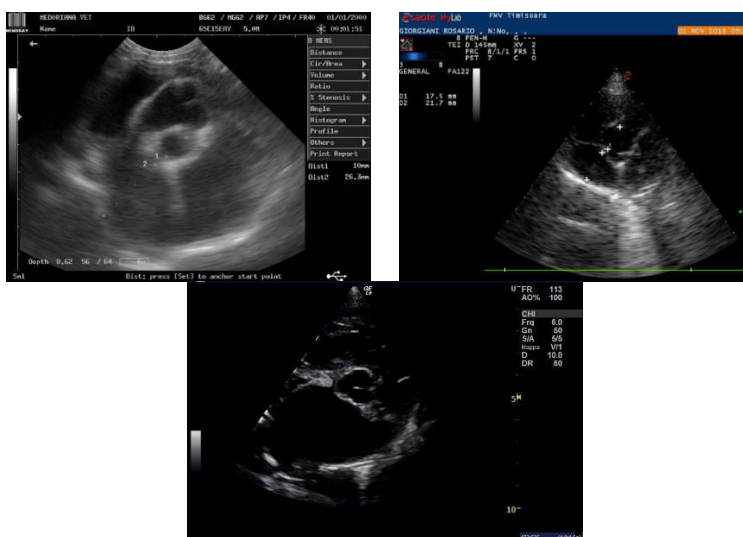


Fig. 1. Echocardiography (mode B, right parasternal window, short axis) in the dog (original)

The enlargement of the mitral ring favors the regurgitation of blood from the left ventricle in the opposite direction, to the left atrium during the ventricular systole, which leads to severe hemodynamic disorders, with the morphological impairment of the heart, without the possibility of returning to the physiological aspect, characterized by a turbulent aspect of the transmitral flow (Fig. 2 - 3). In physiological conditions, at the level of the mitral or tricuspid ring, through color Doppler examination, the blood flow has red or blue appearance.

In case of persistence of cardiac morphofunctional disorders compensatory or dystrophic changes occur. From an evolutionary point of view, the dystrophic aspect of the myocardium implies a myogenic change, characterized by eccentric hypertrophy accompanied by dilation, which favors the increase of the cardiac activity volume. The echocardiographic examination (modes B and M) shows the thickening of the intraventricular septum, with the significant reduction of the left ventricular cavity, resulting in a decrease of the minute volume and sinus tachycardia (Fig. 4 - 5).



Fig. 2. Echocardiography (color Doppler mode, right apical section) in dogs (original)



Fig. 3. Echocardiography (color Doppler mode, apical right section) in dogs (original)

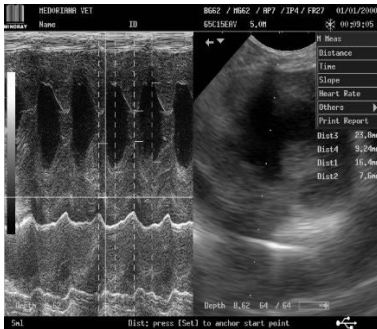


Fig. 4. Echocardiography (B + M mode, right parasternal window, short axis) in dogs with cardiac hypertrophy (original)



Fig. 5. Echocardiography (mode B, right parasternal window, short axis) in dogs with cardiac hypertrophy (original)

In Fig. 6 - 8 are highlighted the echocardiographic aspect of valvular thickening and changes regarding the endocardial architecture (dilatation of the mitral ring, ruptures of the tendon cartilages).



Fig. 6. Echocardiography (mode B, right parasternal window) in dog endocardiosis (original)



Fig. 7. Echocardiography (mode B, right parasternal window, long axis) in dog endocardiosis (original)



Fig. 8. Echocardiography (mode B, right parasternal window, short axis) in dog endocardiosis (original)

By echocardiography (Doppler mode), in the advanced stages of endocardiosis, were highlighted aspects of valvular regurgitation, characterized by the presence of a turbulent blood flow, with shades of red, green, orange (Fig. 9).



Fig. 9. Echocardiography (Doppler mode) in dogs with valve regurgitation (original)

In a Pekinez dog, male, 11 years old, with obvious cardiac symptoms (night cough, intolerance to minimal efforts), by echocardiography (B + M and Doppler mode) was found the presence of a slight left atrial cardiomegaly, but with morpho-functional aspect, normal of both the interventricular septum and the free wall of the left ventricle. When examined in the pulsed Doppler mode, it is noted that the diastolic pattern of the left ventricle is slightly affected, by increasing the degree of rapid filling, high amplitude of the A wave, respectively an increased value of the isovolumetric relaxation time (TRIV), (Fig. 10).

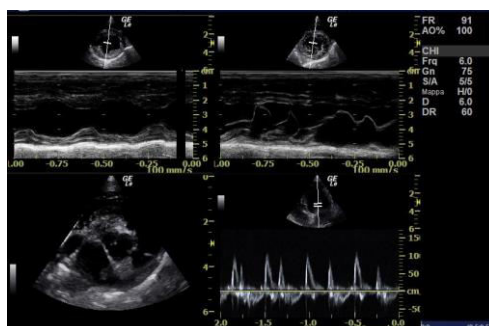


Fig. 10. Echocardiography (mode B, B + M and pw Doppler) in dogs (original)

Table 1 shows the statistical indices and the average of the blood biochemical parameters studied in dogs with endocardiosis.

Table 1

Mean and statistical indices of TGO, CPK, LDH enzymes and T troponin

Specification	Mean	Medium mean error	Standard deviation	Minimum value	Maximum value
TGO (u/L)	32.79	3.60	13.47	15.00	60.00
CPK (U/L)	132.50	9.86	36.88	68.00	190.00
LDH (u/L)	174.80	16.50	61.5	63.00	285.00
T troponin (pg/nm)	-	-	-	<3	>3

Determination of the enzymes creatinine phosphokinase (CPK) and lactate dehydrogenase (LDH) is important because their changes are associated with ischemic and / or degenerative myocardial disorders detectable from early stages.

The blood biochemical examination shows that the TGO enzyme has an average value of 32.75 u/L, with a maximum of 60 u/L and a minimum of 15 u/L, values that fall within the physiological limits. Given that this enzyme does not have a marked specificity regarding myocardial disorders, in the experimental group of dogs with heart diseases, from this point of view, no obvious degenerative changes were registered, because endocardiosis is not always associated with myocardial cell damage.

From a statistical point of view, the maximum CPK value was higher than the physiological maximum limit (190 u/L), but the average of the values recorded in the 14 dogs with cardiac manifestations was increased (132.5 U/L), which implies the presence of some subendocardial and / or myocardial lesions.

Lactate dehydrogenase (LDH) had a maximum value that exceeded the physiological limit (285 u/L), but the average was within the physiological reference range, which confirms that in dogs in the experimental group with heart conditions, there were individuals with phenomena of myocardial ischemia against a background of muscular and endocardial degeneration.

The graphical analysis (Fig. 11) regarding the values of the blood biochemical parameters shows that the TGO enzyme did not exceed the physiological limits, only 3 dogs had a tendency towards a maximum value of this indicator. Regarding serum CPK, a number of 8 dogs recorded pathological values, and the LDH recorded values above the physiological limit in only 3 dogs, from the experimental group.

All these aspects reinforce once again the practical utility, both diagnostic and prognostic of these blood biochemical indicators.

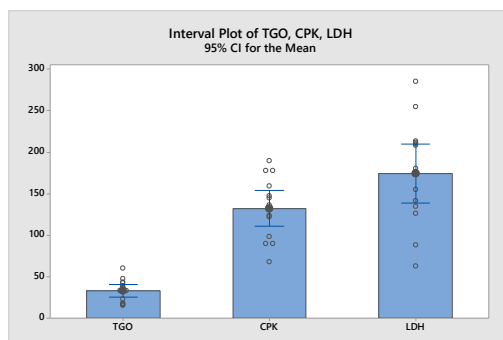


Fig. 11. Graphical representation of TGO, CPK, LDH in dogs with endocardiosis

The mean values and statistical indices of troponin T were in two intervals, respectively up to 3 pg / nm and above this interval (physiological value is 0 pg / nm). This biochemical blood indicator, by clinical point of view is useful in detecting morphostructural changes of the left ventricular myocardium. The results regarding troponin T showed values below and above 3 pg/nm (Table 1, Fig. 12).

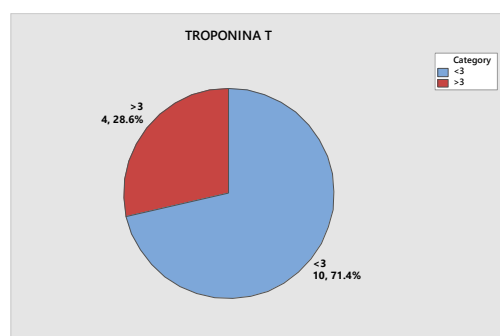


Fig. 12. Graphical representation of troponin T intervals in dogs

The graphical analysis of troponin T intervals showed that only 4 dogs had values above 3 pg / nm (28.6%), while in the other 10 dogs (71.4%), this parameter was within the reference range.

Conclusions

Echocardiography in B and B + M mode is recommended in assessing the morpho-functional integrity of the myocardium and endocardium.

Doppler echocardiography is useful in assessing the diastolic function of the left ventricle.

Through Doppler mode echocardiography can assess the speed of blood flow to the ventricular myocardium and the morphostructural changes of the heart.

The blood biochemical examination shows that glutamate-oxalacetate transaminase (TGO) has an average value of 32.75 u/L, which is within the physiological limits, and is not conclusive for the assessment of cardiac degenerative aspects.

The maximum value of creatine phosphokinase (CPK) was higher than the maximum physiological limit (190 u/L), but the average of the values recorded in the 14 dogs with cardiac manifestations was increased (132.5 U/L), which is useful in assessing the subendocardial and / or myocardial lesions.

Lactate dehydrogenase (LDH) recorded a maximum value that exceeds the physiological limit (285 u/L), an aspect that confirms that in dogs in the experimental group with heart conditions, there were individuals with phenomena of myocardial ischemia, possibly due to muscular and endocardial degeneration.

The graphical analysis of the values of troponin T intervals revealed that only a number of 4 dogs recorded values above 3 pg/nm (28.6%), while in the other 10 dogs (71.4%), this parameter it was within the reference range, being useful in predicting heart conditions.

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RESEARCH ON BOOSTING THE NON-SPECIFIC IMMUNITY IN GALLINACEAE

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Summary

The research aimed the stimulation of non-specific immunity in chickens vaccinated against infectious avian bursitis, after the administration of a probiotic (Poultryzyme TM250). The assessment of the immunomodulatory effect of the probiotic was made following the highlighting of the most important changes regarding the dynamics of nonspecific immune effectors: the leukocyte formula, the lysozyme concentration and the serum properdin. Research has confirmed the beneficial effect of this probiotic, both in birds administered as such and in groups of chickens that have been administered in combination with an immunogen, namely the vaccine against avian infectious bursitis.

Keywords: probiotic, chicken, the average daily gain, specific intake

Probiotics are represented by strains of living microorganisms, selected and concentrated with multiple effects and actions, including the achievement of improved production parameters and potentiation of immune mechanisms. They are real alternatives to the use of antimicrobial feed additives and antibiotics that have a number of disadvantages, among which can be mentioned the destruction of normal flora, the dysmicrobism and the registration of opportunistic infections, in addition to the resistance of bacteria to antibiotics (1, 5, 6, 7, 9, 10).

Although the word „bacterium” is commonly associated with pathogens and disease, probiotics are bacterial strains that help the body function normally and sometimes even prevent the disease. The use of probiotics has been shown to have remarkable effects in stimulating the immune status of animals in general and young animals in particular. Among the beneficial effects it is mentioned the predigestion of some antinutritive factors and even the existence of some antifungal effects (2, 3, 4, 7, 9).

Materials and methods

The experiments were performed on 80 chickens, Ross hybrid broilers, kept in the same microclimate conditions and fed with the same feed. The chickens under study were grouped into four experimental groups (M, I, II and III). We mention that the population was made at the age of one day and the growth was completed at the age of 56 days. All chickens were fed with standard combined feed, respectively in control group and experimental group II feed only, while in experimental groups I and III, the feed was administered together with the probiotic Poultryzyme TM₂₅₀, in an amount of 250 ppm.

Poultryzyme TM₂₅₀ is a probiotic with the following composition: dry yeast culture; gluten and corn syrup; wheat embryos; dry soluble parts: from fish, from the fermentation of *Aspergillus spp.*, *Streptomyces spp.* and from the distillation of maize; dehydrated pancreatic extract and Yuka and Schiderga extract. In order to stimulate their immunity (specific and non-specific), the chicks from two groups, respectively II and III, were immunized against infectious avian bursitis on the 12th day of life.

To determine the immunomodulatory effect of the probiotic Poultryzyme, the main haematological changes were determined, including the concentration of lysozyme and serum properdin.

Haematological and serological tests were performed on blood samples taken from chickens in each group, as follows: on the day of vaccination (R1), one week after vaccination (R2), then two weeks after vaccination (R3) and three weeks after vaccination (R4). We mention that from each chicken, from the four lots studied, two samples were collected, respectively one on anticoagulant (1% EDTA) and the other without anticoagulant. The samples taken were processed in the Immunology laboratory.

To determine the immunomodulatory effect of the probiotic on nonspecific immunity were determined: the leukocyte formula, the lysozyme and serum properdin. The leukocyte formula was determined from blood samples taken on anticoagulant and then from it, smears were made, stained by the May-Grunwald-Giemsa method.

The lysozyme was determined with a classical test, respectively the simple radial diffusion test in 2% agarose gel in which a culture of *Micrococcus lysodeicticus* was incorporated. We mention that the interpretation of the test takes into account the fact that the diameter of the lysis halo of the germs included in the medium is directly proportional to the concentration of serum lysozyme.

Serum properdin was dosed according to a method taken from the "Pasteur" Institute in Bucharest. The principle of the method consists in the colorimetric determination of properdin isolated by complexation on inulin and treated with Biuret reagent.

During the experimental period, all chickens, from the four groups, were verified daily, monitoring their general condition and mortality.

Results and discussions

According to data from the literature, immunological reactivity in chickens is conditioned by the morphological and functional integrity of the immune system, in which the Fabricius bursa plays an extremely important role.

In the experiment performed by us, the main monitored parameters highlighted relevant values and the results were centralized and systematized in tables. Results obtained from hematological investigations (leukocyte formula) are presented in Table 1.

Table 1

The effect of the probiotic Poultryzyme TM250 on leukocyte formula (average values)

Group	Harvesting	L	N	E	B	M
M	R ₁	62.1	30.5	3.1	0,7	3,6
	R ₂	59.9	33.3	2.3	1.1	3.4
	R ₃	64.1	28.9	2.5	1.0	3.5
	R ₄	65.9	27.4	2.9	0.9	2.9
I	R ₁	62.1	31.2	3.1	0.9	2.7
	R ₂	60.9	33.1	2.8	1.0	2.2
	R ₃	68.4	25.4	2.4	1.2	2.6
	R ₄	69.5	24.3	2.2	0.9	3.1
II	R ₁	60.8	32.2	2.5	1.0	3.5
	R ₂	64.4	28/2	2.7	1.2	3.5
	R ₃	67.3	25.6	2.7	1.1	3.3
	R ₄	69.4	23.8	2.7	1.0	3.1
III	R ₁	60.1	33.3	2.9	0.9	2.8
	R ₂	67.5	26.1	2.6	0.7	3.1
	R ₃	69.3	24.2	2.5	08	3.2
	R ₄	70.4	23.2	2.2	1.0	3.2

The results on the leukocyte formula confirm that the number of lymphocytes increased in all four experimental groups, an increase being progressive and more evident in experimental groups I and III, that received simultaneously standard and probiotic feed. Analyzing the average values (table 1) was found that the maximum values were recorded three weeks after vaccination, but no significant differences were found between the three experimental groups, noting that the lowest values were found in the control group (unimmunized and who was not given a probiotic).

Instead, analyzing the results recorded in the two vaccinated groups, it can be seen that the highest values were recorded in experimental group III, which was also administered probiotically. We mention that the marked lymphocytosis, in the case of experimental groups, may be a consequence of the influence of the components of the immunomodulatory preparation, which also had the effect of stimulating the Fabricius bursa.

Analyzing the evolution of the post-vaccine reaction, in terms of non-specific immunological parameters, in these groups of chickens under study, we found significant differences from one group of chickens to another and from one immunological parameter to another.

Thus, regarding lysozyme, the highest values were recorded in the vaccinated groups, experimental II and III, with the mention that the maximum value 55.15 $\mu\text{g} / \text{cm}^3$ was recorded in the last experimental group (III) at 21 days of age at the time of vaccination (Table 2). Also, higher values were recorded in the first experimental group (I), unvaccinated group but which received probiotic food, which confirms the immunomodulatory role of the probiotic in relation to the immune system (Fig. 1).

Table 2

The average values of serum lysozyme

The determined parameter	Group	Harvesting			
		R1	R2	R3	R4
Lysozyme ($\mu\text{g}/\text{cm}^3$)	M	10.15	11.90	13.80	15.05
	I	12.95	19.00	26.10	25.30
	II	15.05	39.50	32.00	31.30
	III	22.70	42.10	48.30	55.15

Serum protein showed a similar dynamics with the mention that the values recorded in the vaccinated groups were quite close to each other, but significantly higher than in experimental group II, group that did not receive the immunomodulatory preparation in food. From the recorded data, there is an increase in the concentration of properdin, from 16.18 mg/100ml serum to 32.07 mg/100ml serum, after two weeks after vaccination in the case of experimental group III, with the mention that the maximum value was recorded in experimental group III, after three weeks after vaccination (36.03 mg/100 ml serum), a value close to that found in experimental group II. It can also be seen that 21 days after vaccination the values recorded in groups II and III remain quite close (Table 3, Fig. 2).

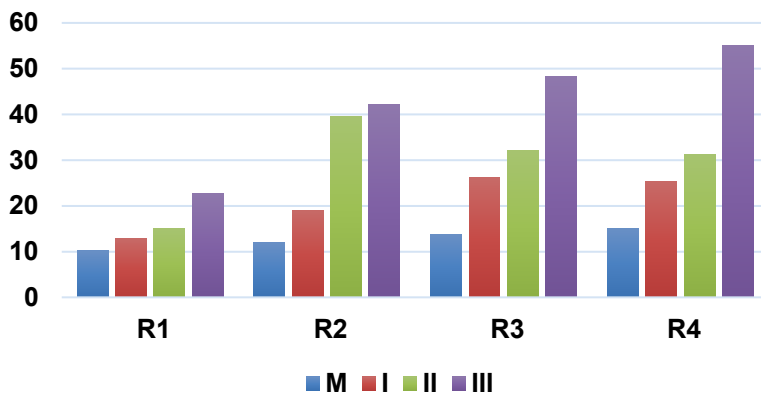


Fig. 1. Average values of serum lysozyme at the chickens under study

Table 3

The average values of serum properdin

The determined parameter	Group	Harvesting			
		R1	R2	R3	R4
Properdin (mg/100 ml serum)	M	17.04	16.93	16.18	19.04
	I	18.35	20.17	27.05	29.98
	II	22.05	23.45	29.05	35.68
	III	21.95	24.81	32.07	36.03

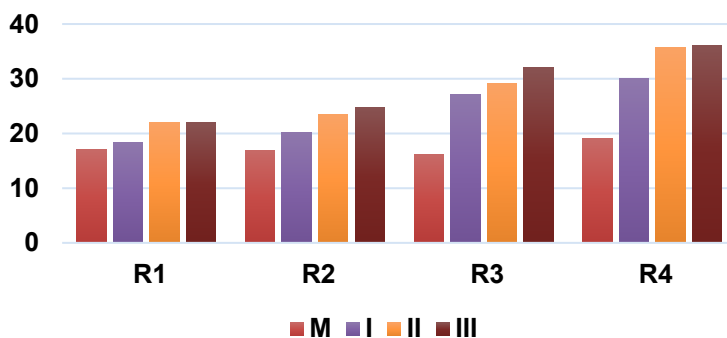


Fig. 2. Average results of the serum properdin in the chickens under study

Considering the results obtained, in the group in which probiotics were administered, both in terms of the concentration of lysozyme and serum properdin and the increase in the number of lymphocytes, we consider that the product stimulates the immune reactivity in chickens.

Conclusions

Hematological tests showed a progressive increase in the number of lymphocytes at 14 and 21 days after vaccination, the maximum values being recorded in the group where the vaccine was administered together with the probiotic (group III).

The probiotic Poultryzyme TM₂₅₀ stimulates the effectors of nonspecific immunity, the results especially the production of lysozyme and to a lesser extent the production of serum properdin.

Compared to experimental group II in which no Poultryzyme TM₂₅₀ was administered, in experimental group III in which it was administered, the average values of the concentration of nonspecific immune effectors were higher in all harvests.

The results obtained prove the immunomodulatory effect of the probiotic Poultryzyme TM₂₅₀ on both the humoral and cellular immune response.

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