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ŞI MEDICINĂ VETERINARĂ A BANATULUI
TIMIŞOARA**

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BACTERIOLOGICAL STATUS OF CHICKEN MEAT IN WESTERN ALGERIA

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Summary

This study was conducted to evaluate the microbial quality of fresh chicken meat retailed at Tiaret City in Western Algeria. Randomly samples (70) were taken from chicken breast and thigh (35 of each) during 2019. Standards analysis was carried out to asses aerobic plate count (APC), *Staphylococcus aureus*, Escherichia coli and Salmonella, Expressed in log CFU/g, the average load of APC, S. aureus and *E. coli* counts were 5.8, 4.09 and 4.89 respectively. Salmonella spp was found in 31.42% of the examined samples. The breast was more contaminated than the thigh. Improving good hygiene practices allows the reduction of the risk.

Keywords: Chicken meat, Aerobic Plate Count, *S. aureus*, *E. coli, Salmonella*

In Algerian, about 340,000 tons of white meat and 4.8 billion eggs are produced annually. However, this sector, represented by 20,000 farmers employing about 500,000 people continues to import the majority of food and other inputs corresponding to 80% of 2,500,000 tons of feed (1).

With high levels of low-cost animal protein, chicken is considered as a major food in human nutrition (14). It is also a healthy food regarding its low fat and cholesterol content (27). The recent increase in the consumption of meat and its products arises from reasons including high protein contents, vitamins, minerals, lipids and savory sensation (31).

The bacteria in food can be pathogen while some others, when present in huge amount may cause economic losses due to the defects in food organoleptic characters (42).

Thus, chemical and physical factors can alter chicken meat depending on the microflora affecting the content by the conditions of slaughter, disinfection, and storage (5).

Transformation operations increase the surface area of meat in contact with working surfaces and air. Consequently, the level of bacteria is higher in transformed products than on primary cuts (2).

Cross-contamination between carcasses or cuts may occur by direct contact or through contact with contaminated surfaces (49).

It was reported chicken consumption was the first cause of foodborne outbreaks in the USA between 1998 and 2012 (11).

Centers for Disease Control (CDC) reported foodborne diseases cost the United States between 5 and 6 billion dollars annually in direct medical care and

lost productivity (10). Moreover, in developing countries where the cold chain is rare and worker supplies generally soiled, theses illnesses may lead to billion dollars in illness and 4-6 million deaths every year (9).

Chicken meat quality is assessed by determining microbial and chemical parameters (8). The aerobic plate count (APC) is used as an indicator of the level of bacteria in meat and is a useful tool in monitoring food safety (7). Besides, the coliform group of bacteria is a reliable indicator of fecal pollution, improper handling, and storage of meat and meat products while salmonella contamination may occur at any stage (16, 51).

Therefore, this work was undertaken to assess the bacteriological quality of fresh poultry meat marketed at Tiaret city, western Algeria.

Materials and methods

Seventy samples of fresh chicken meat were randomly collected from butcheries, during 2017-2018. The samples were divided into two groups of 35 samples. The first group was collected from the breast and the second from the thigh of the same chicken. The samples were thereafter transported directly to the laboratory under cold and aseptic conditions. They are subjected to the same microbial laboratory examinations.

Twenty-five grams of each sample meat was transferred to aseptic blender jar filled with 225 ml of 0.1% sterile peptone water. Each sample was then crushed to provide a homogenate, from which tenth-fold serial dilutions were prepared (25). The prepared samples were subjected to the following analysis:

- Determination of Aerobic Plate Count (4).
- For detection of *S. aureus*, 0.1 ml of the homogenate was streaked on Mannitol Salt Agar (MSA). The plates were incubated at 37°C at 24-48 h. Then the yellow or white colonies grown were examined by biochemical tests such as gram staining, catalase and coagulase test (26).
- E. coli was isolated by using MacConkey broth and Eosin Methylene Blue (EMB) agar (3).
- Salmonella: 1 ml of the pre enriched broth were streaked aseptically into Xylose Lysine Desoxycholate agar plates and incubated at 37°C for 24 h. The presumptive identification of Salmonella was done based on morphology and color of the colonies on the culture media (24).

The confidence interval 95% of prevalence rates of microbes in the minced meat was estimated using an exact binomial distribution. The average logarithms of germs content in the chicken meat were compared using unequal variance Welch test (40).

Results and discussions

Table 1 summarizes overall bacterial results expressed in log CFU/g except for *Salmonella* where presented in percentage.

Table 1

Overall results expressed in log CFU/g

Germs	Meat	Mean (±Sd)	Mean (±Sd)	Min	Max	Standard**	P value (unequal variance Welch's test)
APC	Thigh	5.80 (0.55)	5.33* (0.26)	4.28	5.59	N. D.	0.239133e-21*
	Breast		6.27 (0.31)	5.26	6.68		
S. aureus	Thigh	4.09 (1.59)	3.87 (1.49)	0	5.42		0.239133
	Breast		4.31 (1.62)	0	5.41	4	
E. coli	Thigh	4.89	4.71 (0.43)	4.14	5.53		0.0017*
	Breast		5.07 (0.47)	3.2	5.7	4.69	
Salmonella (presence)	Thigh	31.42 %	25.71 (%)			Absent/ 10g	
	Breast		37.14 (%)				

* Significant difference at 95% confidence interval; **J.O.R.A. N° 39. Arrêté interministériel du 4 octobre 2016 fixant les critères Microbiologiques des denrées alimentaires

A raw food is always contaminated by initial contamination and the environment but also while handled.

Aerobic Plate Count (APC) gives an idea of hygienic measures applied during processing and helps in the determination of the keeping quality of the poultry carcasses as well. Thus, it is the most reliable method for the detection of the sanitary levels of proper processing, storage, and marketing of food products. It was considered a general criterion for evaluating microbial contamination and hygienic conditions in treatment plants (14).

The overall load in APC was 5.80 log CFU/g while individual contamination varied from 4.28 to 6.68 log CFU/g depending on the sampling area (Table 1). However, the contamination is significantly higher ($P<0.05$) in the breast (6.27 log CFU/g) compared to the thigh (5.33 log CFU/g).

Knowing that national standards are absent for APC in chicken and as in several countries the limit is 5 log CFU/g, we concluded that 83.57% of the samples exceed admitted regulatory thresholds.

However, overall results are within the limits reported by Banana et al. (6) with the microbial load ranged from 2 to 6 log CFU/g.

The total APC load (5.33 and 6.27) is inferior to that (6.38 log CFU/g and 6.56 log CFU/g) reported by Saikia and Joshi (43) and (6.64 and 6.57) found by

Hassanien et al. (21) in thigh and breast respectively while a load of 8.6 log CFU/g was reported by Nossair et al. (39).

However, Daoud et al. (15) recorded lower values: 3.43 log CFU/g for thigh and 3.32 log CFU/g for breast while Shaltout et al. (46) and El Taher-Amna (17) revealed 4.55 log CFU/g and 4.91 log CFU/g respectively in raw chicken meat.

Other studies reported lower count: 5.23 log CFU/g (20); 4,39 log CFU (33); 5.07 log CFU/g and 4.94 log CFU/g (23); 3.6-6 log CFU/g (13); 5.01 log CFU/g (19); 5.23 log CFU/g (32); 6.18 (12); 5.06 (28).

In breasts with skin, Kozačinski et al. (31) and Saleh et al. (44) reported 3.67 log CFU/g and, 4.4 log CFU/g respectively.

Comparing with the APC recorded in other studies, our results highlight that the overall hygienic quality of studied chicken meat was below standards.

The APC load depends largely on many factors knowing that cross-contamination is important and crucial not only during the slaughtering process but after that as well. Keeping the carcass at a higher temperature helps bacteria growth. Some habits like insufficient hand washing and the inadequate use of material (e.g. knives, chopping tables) participate in increasing the meat bacterial load.

Staphylococcus aureus is a common food born pathogenic bacteria isolated from raw and undercooked chicken meat and its consumption may lead to the infection and/or toxicity in consumers (22).

The average load of *S. aureus* was 88.57% of the samples with an average load of 4.09 log CFU/g.

Other high occurrences were reported: 78.48% (6) and 100% (50) while inferior values have been recorded: 24% (46), 46.66% (19), 30.30% (32), 16.66% (29), 27.5% (36) and 28.75% in samples of breasts with skin (31).

As for other germs, the breast remains more contaminated (4.32 log CFU/g) than the thigh (3.84 log CFU/g) close to the result (4.79) recorded by Javadi and Safarmashaei (28).

Results lower than ours were obtained elsewhere: 2.1 log CFU/g (20), 1.08 log CFU/g (19), 1.53 CFU/g (48) and 2.67 log CFU/g (29).

In chicken breasts with skin: Chaiba et al. (12) reported 2.43 log CFU/g and 2.98 log CFU/g (31).

In freshly slaughtered chicken carcasses, staphylococcus mean value was of 5.39 log CFU/g (39) while Mohamed et al. (36) reported 4.45 log CFU/g.

Poultry food products are important sources of *E. coli* because at the time of slaughter, fecal contamination from the intestines contaminated the carcass. As a result, poultry meat can be contaminated with fecal material or ingesta and with bacteria associated with these contaminants (19).

The *E. coli* overall load was of 4.89 log CFU/g higher than that (2.60 to 4.33) reported by Alvarez-Astorga et al. (2) and less than the 3.57 log CFU/g and 4.4 log CFU/g reported by Eye and Arslan (18) and Manguiat and Fang (34) respectively.

The zoonotic potential of *E. coli* from chicken is very serious since it can cause serious illness (35).

Salmonella is a human pathogenic bacterium that can contaminate the gastrointestinal tract of birds so, it is highly important to detect its presence (42).

The overall incidence rate of salmonella is of 31.42%, consistent with the 30% reported by Suleiman et al. (47). These results are largely inferior to 56% and 50% reported by Samaha et al. (45) and Guergueb et al. (19) respectively. Moreover, Wilfred Ruban et al. (50) reported 65.71% *Salmonella* in breast and 71.43% in the thigh in non-sophisticated outlets, similar conditions as in our study.

On another side, lower *Salmonella spp.* contaminations were observed elsewhere: 21% in Ethiopia (37), 9.52% of chicken breasts (31), 22.6% (30). However, no *Salmonella* was recorded by Mohamed et al. (36).

These differences in amount of contamination are related to hygiene respect and procedures undertaken.

In this sense, Nidaullah et al. (38) reported a high prevalence of *Salmonella spp.* in carcasses due to cross-contamination throughout the various stages of processing at wet markets. Rivera-Perez et al. (41) also investigated the risk points during broiler carcass processing and reported that Salmonella contamination increased from 10% to 40% during evisceration and subsequent spray washing.

Comparison of results of the bacteriological analysis of chicken breasts without and with skin shows that fillets contained a higher number of *Salmonellae*, as well as *S. aureus* and *L. monocytogenes* (31).

The higher bacterial load of examined freshly slaughtered chicken carcasses samples may be attributed to the fact that live birds harbor large numbers of bacteria on their feathers, feet, and feces in addition to cross-contamination.

Conclusions

From the result obtained in the present study, one may conclude that examined chicken samples marketed in Tiaret City had unsatisfactory conditions which may be attributed to numerous conditions as transportation, slaughtering, handling, distribution, and storage. The examined chicken meat with variable but relatively high load in aerobic plate count, *S. aureus*, *E.coli* and the presence of salmonella render them unfit for human consumption. These germs also represent a public health hazard.

There is a need to improve good hygiene practices to allow risk reduction.

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FUNGI AND BACTERIA LOAD IN AIR OF A POULTRY PRODUCTION SYSTEM

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Summary

It is recognized that the air quality of the animal and hens houses, represented by physical, chemical and biological parameters, can influence their production performance. Workers (cares, managers, herdsmen, veterinarians) in hen's houses, who spend most of their time in buildings, thus being subjected to the strongest exposures, are at high risk. The occurrence of several fungal species in the environment appears to be related to human hypersensitivity disorders. Farmer's lung disease, asthma, poor production performance and reduced disease resistance of poultry and farm animals are certainly issues that may be related to the presence of powders, harmful gases and microorganisms in the air of shelters. The study aimed to determine the total number of fungi and the presence of species with allergic potential as well as the total number of aerobic mesophilic germs using gravitational sedimentation method. Increased values of the total number of fungi and the total number of aerobic mesophilic germs were found in the study shelter. The species of fungi recognized as potential allergens (*Aspergillus, Alternaria, Cladosporium* and *Penicillium*) were highlighted in an increased proportion in this study. The large proportion of *Aspergillus* species presents a particular risk for the development of aspergillosis in the caring staff and in the birds in the shelter.

Keywords: fungi, bacteria, poultry house

The biological factors of the microclimate in the animal shelters, in the strict sense of the term, are represented by the microorganisms in the air of the shelters. Due to the close relationship between the microorganisms and dust particles, that constitute the carrier and the vehicle of germs in the air, in the category of biological factors are also include the dust particles on which the microorganisms closely adhere to, especially particles that are alive (bacteria, viruses, fungi, spores and fragments of germs) (5, 7, 15).

It is recognized that the air quality of the animal and bird shelters, rendered by physical, chemical and biological parameters, can influence their production performance. The effects of air quality on animals and birds represent an economic problem in particular. In addition, poor air quality is also transposed by endangering the health of workers in shelters (11).

Farmer's lung disease, asthma, poor production performance and reduced disease resistance of poultry and farm animal s are certainly issues that may be related to the presence of dust, harmful gases and microorganisms in the air of shelters (8).

The air quality inside the bird shelters can and should be controlled to protect both the health of the workers and the birds they care for (13). Otherwise, respiratory distress, aggravated by exposure to improper environments, may occur. Very few bird diseases can be transmitted to humans, but situations where transmission is possible should be avoided. Workers (cares, managers, herdsmen, veterinarians) that work in bird complexes (hens shelters) and spend most of their time in buildings, thus being subjected to the strongest exposures, are at high risk (4, 10, 16).

Materials and methods

The study was conducted in a poultry house on a farm located near Timisoara, during April and May, 2018. The bird hall has a length of 55 m, a width of 18 m and a height of about three meters.

The hall was populated with chickens from the Isa Brown breed. The period of the determinations made ranges from 49 to 56 weeks after the population. The birds are reared on permanent litter, with an average density of 10 birds to a square meter. The ventilation in the shelter is artificial, made by 10 fans, of which five are arranged on the longitudinal walls and one on each on the ends of the hall.

The study aimed to determine the total number of fungi (TNF) and the presence of species with allergic potential as well as the total number of aerobic mesophilic bacteria (TNAMB). The two indicators were determined by gravitational sedimentation method.

For this method were used sterile Petri dishes, with a diameter of 9.5 cm. Nutrient agar was used to determine the total number of germs, and the Sabouraud medium with antibiotic addition was used to determine the total number of fungi.

The determinations were made for two months, April and May, once a week, at the same time, between 9:00 and 10:00. The determination of the hour of making the determinations took into account the program that is carried out in the hall, before the fodder is carried out. Five places were chosen for the determination:

- at the entrance to the shelter, on the right side;
- at the entrance to the shelter, on the left side;
- in the middle of the shelter;
- at the end of the shelter, on the right side;
- at the end of the shelter, on the left side.

The determination of the two indicators (TNAMB and TNF) by the gravitational sedimentation method consisted of exposing the solid culture media, distributed in Petri dishes, in the established places, for a different time. In the case of TNAMB determination, the exposure time was 2.5 minutes at the first determinations and then one minute at the other determinations, as a result of the large germ load in the shelter. To determine the total number of fungi, the plate

exposure time was 10 minutes.

After the exposure, the plates were brought to the laboratory and incubated at thermostat at 37°C, 24 hours, for TNAMB and at 25°C temperature, plates for total number of fungi, for 7 days. After the number of colonies on each plate was read, the number of microorganisms (mesophilic aerobic bacteria and fungi) was calculated to obtain the microorganism load in one cubic meter of air. For this was used a specific by formula:

$$N - (germs/m^3) = \frac{63662xn}{d^2xt}$$

N = number of germs/m^3 air;
n = the number of colonies on the entire Petri plate;
63 662 = the empirical coefficient, derived from Omeleanski's finding;
d = plate diameter in cm; t = exposure time in minutes.

To determine the proportion of fungi species with allergic potential, each colony from the plates was microscopically examined using aniline blue solution for staining (14).

In the shelter were also determined temperature, relative humidity of the air as well as the velocity of the air currents, using a multifunctional device.

Data were centralized and statistically processed using the Excel program.

Results and discussions

Table 1 shows the values of the total number of fungi in the air of shelter taken in the study obtained by the sedimentation method.

During the study period, the total number of fungi in the shelter ranged from 4562.4 m^{-3} to 81769.3 m^{-3}. There is an increase in the total number of fungi in the air in the second half of the study period, more precisely in the last two weeks, when the temperature was on average 27.7ºC, and the relative humidity of the air on average 65.3% (Table 2). Thus, the average value of the total number of fungi increased from 7643.2±19.2 m^{-3} at the beginning of the study period (02.04.2018), to 53185.9±37.7 m^{-3} at the end of the period (14.05.2018), representing an increase of about seven times.

The increase of the fungal concentration inside the living spaces, as well as the animal shelters, during the summer and autumn period, was also reported by other researchers. Gomez et al. (9), reported the presence in high concentrations in homes of fungi, that are recognize with increased allergic potential, during the autumn (73%) and summer (64.3%) respectively. Of the four genera identified in Gomez et al. Study, *Cladosporium* was present in the highest proportion, followed by *Penicillium* (78%), *Aspergillus* (71%) and *Alternaria* (46%).

Table 1

The values of the total number of fungi in the air of hens shelter taken in the study obtained by the sedimentation method

Data	Total number of fungi (cfu/m^{3}air)					$\overline{X} \pm cv$
	entrance of the shelter, on the left side	entrance of the shelter, on the right side	in the middle of the shelter	end of the shelter, on the left side	end of the shelter, on the right side	
02.04.	5682.2	9563.6	7563.5	8452.6	6954.3	**7643.2 ± 19.2**
09.04.	9657.2	6748.7	**4562.4**	8563.1	5632.5	7032.7 ± 29.6
16.04.	9684.9	7121.3	9514.6	9632.4	6756.7	8541.9 ± 17.2
23.04.	9654.4	9056.3	10056.4	13563.7	9061.1	10278.3 ± 18.3
30.04.	10105.6	9865.6	9978.4	11198.7	10084.1	10246.4 ± 15.2
07.05.	7049.1	10226.1	11190.4	15507.9	9163.8	10627.4 ± 29.4
14.05.	28901.2	60622.1	40884.6	53752.4	**81769.3**	**53185.9 ± 37.7**

Table 2

The values of temperature and relative humidity of the air during the study period

Place	Physical parameters of the air during the study period:													
	Tempreture ºC							Humidity %						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Outside the shelter	14.8	15.6	17.8	21.3	25.6	28.2	27.5	66.3	65.7	65.2	44.8	46.1	39.6	45.6
In the shelter	24.2	2.8	25.6	25.8	26.6	27.7	27.8	59.8	59.6	60.1	59.6	62.6	65.3	65.3

On the 35 plates with Sabouraud exposed to determine the total number of fungi, a total of 537,781 colonies grew. Each colony was examined under a microscope using aniline blue staining. Following the examination, eight main types of fungi were identified. Of these, only four genera were considered, which are recognized as having a relevant role in triggering allergies: *Alternaria, Aspergillus, Cladosporium, Penicillium.* The total number of colonies belonging to the four genera was 45,980. After calculating the proportion of each genus, the results presented in table 3 were obtained.

Table 3

The number of colonies and the proportion of the four genera with allergic potential on exposed plates (N = 45980 colonies)

Fungi genus	The number of colonies	The proportion from the total colonies (%)
Alternaria	6530	14.2
Aspergillus	24416	53.1
Cladosporium	5746	12.5
Penicillium	9288	20.2

The obtained results reveal the presence in a large proportion of the species of the genus *Aspergillus*, which represents 53.1% of the total fungi colonies isolated on the exposed plates.

Aspergillus fumigatus colonies were isolated in large numbers from different places in the shelter (Fig. 1).

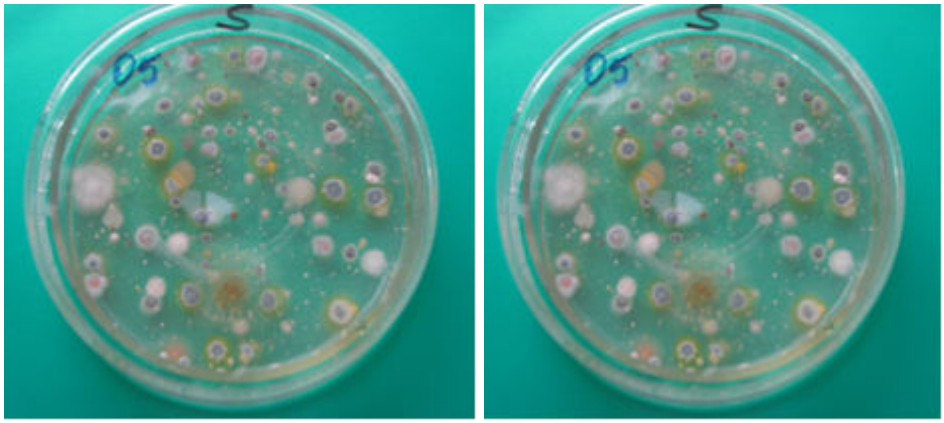
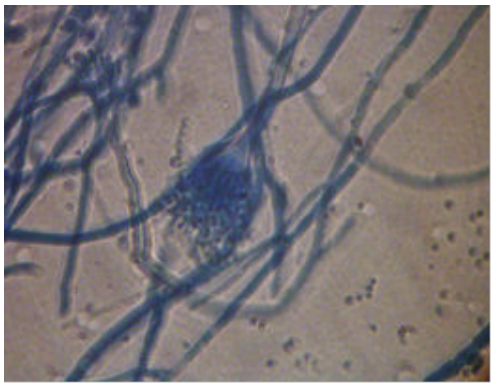
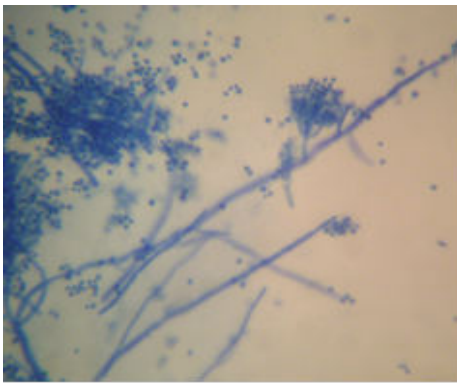


Fig. 1. Colonies of fungi grown on plates with Sabouraud environment exposed in the bird shelter

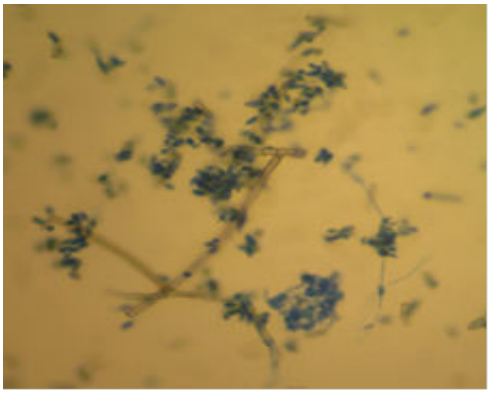
The microscopic examination revealed the structural characteristics of the *Aspergillus fumigatus species* (Fig. 2A), *Penicillium* (Fig. 2B), *Cladosporium* (Fig. 2C) and *Alternaria* (Fig. 2D).



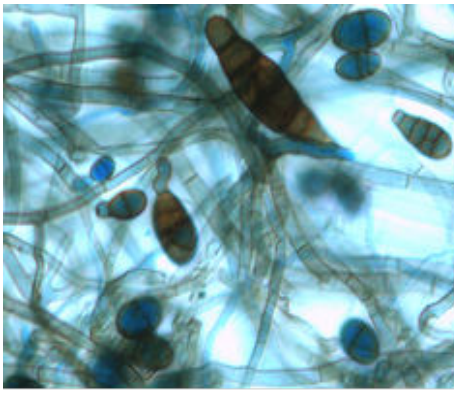
A - Aspergillus fumigatus



B- *Penicillium spp*



C - *Cladosporium spp*



D - *Alternaria spp*

Fig. 2. Microscopic aspect of the main allergenic species isolated from bird shelter

The species of the genus *Penicillium* were present in a proportion of 20.2% (Fig. 2B), and those of *Cladosporium* (Fig. 2C) and *Alternaria* (Fig. 2C) in proportion of 12.5% and 14.3% respectively.

For the concentrations of airborne fungi at the workplace, no recommended levels or limits have been set.

There is no data to attest the concentration limit for *A. fumigatus* that could cause allergic symptoms. However, other limit concentrations for airborne fungi are estimated at 100 spores of *Alternaria*/m^3 air, 3.000 spores of *Cladosporum*/m^3 air (9).

The presence of fungi of the genus *Aspergillus* in high concentration implies a particular risk of aspergillosis both for the caring staff and for the birds.

According to data from the literature, inhalation of an increased amount of spores of *Aspergillus candidus* caused toxic organic dust syndrome in students who moved wheat from a store (8, 11). *Aspergillus versicolor* was identified as the etiologic agent of aspergillary alveolitis (11).

Fungi of the genus *Penicillium* and species of other genera are recognized as agents of fungal allergies (2, 3).

The values of the total number of germs in the air of the bird shelter, taken in the study, obtained from the determinations made by the sedimentation method are shown in table 4.

Table 4

The total number of mesophilic aerobic bacteria (TNMAB) /m^3 in air of the hens shelter by the sedimentation method

Data	Total number of fungi (cfu/m^{3}aer)					$\overline{X} \pm cv$
	entrance of the shelter, on the left side	entrance of the shelter, on the right side	in the middle of the shelter	end of the shelter, on the left side	end of the shelter, on the right side	
02.04.	198934.3	176596.2	201214.6	212981.1	271021.4	212149.5 ± 16.7
09.04.	223407.5	256241.6	298623.5	300213.8	256256.8	266948.0 ± 12.1
16.04.	201112.7	245231.1	276073.2	294125.4	290454.3	261399.3 ± 14.8
23.04.	221895.9	203212.3	256134.2	299314.2	286542.2	253419.8 ± 16.1
30.04.	241213.6	227843.6	238952.4	322114.2	321324.6	270289.0 ± 17.4
07.05.	253773.8	177641.7	155084.1	101509.5	135346.1	164671.0 ± 34.6
14.05.	135139.7	265114.8	234127.4	**440710.4**	364963.3	288011.1 ± 41,1

The total number of mesophilic aerobic bacteria in the poultry shelter during the study period ranged from 176596.2/m^3 to 440710.4/m^3. Similar with the total number of fungi, values of this parameter were also increased during the last period of the study, when the average values obtained (288011.1/m^3) exceeded the values accepted by specialists for the air quality of animal shelters that is 250.000 germs/m^3 (5).

The results of this study are comparable with the data from the literature. A very large number of studies have already been published on this subject, the load of microorganisms from animal shelters, with values ranging from 104 to 106 CFU/m^3 (1, 3, 6).

In practice, the microbial load in air of animal shelters often exceed 10 - 20 times the value admitted by some authors that take in consideration the value of 25.000 germs/m^3 (5).

As the permanent litter ages, the number of fungi spores increases from an average of 8,000 /m^3 air and reaches over 50.000/m^3 air (16).

The situation is incomparably better in the halls with hens kept in batteries,

where the highest average values are 14.000 spores of fungi/m^3 air (16).

The highest concentrations of active fungi and total fungi (but also of bacteria) were detected in bird shelters, compared to shelters of other categories of farm animals. The levels determined depend on the type of sampling method (15).

The air fungi concentrations in the shelters with battery cells (2.700 CFU/m^3 air) were significantly lower than in the other bird growth systems (which had average values between 7.500 and 320.000 CFU/m^3 air) (12).

Conclusions

Increased values of the total number of fungi and the total number of aerobic mesophilic bacteria were found in the air of shelter studied.

The species of fungi recognized as potential allergens (*Aspergillus, Alternaria, Cladosporium* and *Penicillium*) were highlighted in an increased proportion in this study.

The large proportion of *Aspergillus* species presents a particular risk for the development of aspergillosis in the caring staff and in birds.

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CORRELATIONS BETWEEN DIFFERENT PHYSIOLOGICAL STAGES AND THE PREVALENCE OF CALCIUM METABOLISM DISORDERS IN EWES

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Summary

Currently the accuracy and precision of the blood biochemistry techniques allow both the monitoring of the animals health, as well as creating an individual metabolic profile that reflects the nutritional needs of each individual. Thus, metabolic profiling tests complement classical morphoclinical diagnostic methods. The quantitative changes of the main blood constituents are an important factor in establishing a diagnosis, the dosage of the main blood parameters also having a prognostic value, since, even if the general state of the animal is unchanged, disturbances of these values - abnormally low or abnormally increased, can cause disturbances of homeostasis and, implicitly, organic or systemic disorders within a relative time frame. The main objective of this research paper is to detect the influence of the physiological status on the mean values of blood calcium, and, therefore, on the prevalence of the disorders and pathological entities which can derive from that. During this study significant variations of several electrolytes have been observed, the most striking variations of a blood parameter in correlation with the physiological status being noted in calcium. This type of results can be correlated with the high fetal demands during gestation or with the increased milk synthesis during the early stages of the lactation period. Another aspect that should be taken into consideration is the fact that during gestation the ewes' diet is restricted, the animals being fed mostly with wheat bran, in order to prevent the accumulation of gas in the rumen. Regardless of the cause, when the calcium level in the ewe's blood decreases significantly, below the lower limit of the reference range for this species, the hypocalcaemia syndrome occurs, with various pathological implications. The most common implication of the hypocalcaemia in ewes is the occurrence of the parturient paresis, morbid entity caused by the decrease calcium intake along with an increased calcium requirement of the mother's body during late gestation. The most common clinical signs include ataxia, salivation, decreased motility of the rumen, bloating, constipation, and, if it is not treated properly, can even lead to the death of the animal. Therefore, performing regular blood test during high demanding physiological statuses, such as late gestation and early lactation in ewes proves to be extremely important in order to prevent the occurrence of hypocalcaemia and to correctly assess the level of calcium supplementation necessary for each individual.

Keywords: calcium, ewes, parturient paresis, hypocalcaemia

The study of the blood electrolytic profile in ewes in demanding physiological periods, respectively advanced gestation and early lactation, is of particular importance, as any change of these parameters values can lead to a

major disturbance of the homeostasis translated by the appearance of various pathological entities and, implicitly, by the decrease of the productivity (1, 7, 8).

The quantitative changes of the main electrolytes blood concentrations are an important factor in establishing a diagnosis and are the consequence of functional/morphological disorders or of the body overstress in different physiological or pathological stages (5, 6, 10).

Hypocalcaemia represents the most common calcium disorder of ewes and it frequently occurs around parturition and also at the beginning of the lactation period. It can have an important economic impact; therefore the disease prevention is crucial by providing adequate feed and reducing stressful situations, particularly in the critical stages of pregnancy and lactation.

Due to the development of the foetal skeleton, the highest demand for calcium occurs 3-4 weeks prior parturition (2, 3). In the last part of the gestation period the ewe will need to mobilize some of its own skeletal calcium in order to meet the calcium requirement, therefore transitory periods of hypocalcaemia may occur.

Hypocalcaemia is most commonly seen in the late stage of the gestation period and in early lactation, although it can occasionally occur in dry sheep. The disease occurs when the ewe's body fails to mobilise enough calcium from the bones in order to maintain normal blood calcium levels and to meet the high requirements for calcium in these two demanding physiological stages (9).

Parturient paresis can occur at any time from 6 weeks before to 10 weeks after parturition; however, the greatest demand for calcium is thought to occur 1–3 weeks before parturition because of the foetal skeleton mineralization, particularly when multiple foetuses gestations are present (4). As concerning the clinical signs, at the onset of the disease the affected ewes become isolated from the flock and are unable to raise themselves from their knees and assume sternal decubitus. Over two to six hours the ewe becomes dull, weak and unable to stand even when supported. Muscle tremors and rumen stasis may also occur. The rectum is flaccid and may contain pellets of dried faeces. Passive reflux of rumen contents may occur with green fluid present at the nostrils and around the lower jaw. Without appropriate therapy, the condition develops to coma, and death follows 24 to 48 hours after the onset (11, 12). Treatment with calcium borogluconate will result in a rapid recovery, within 15-30 minutes. Low blood magnesium and glucose are also common in ewes affected with hypocalcaemia, therefore treatment with magnesium sulphate and glucose may also be warranted. If the ewe does not respond quickly to treatment it may be necessary be treated for pregnancy toxaemia as this is a common effect of hypocalcaemia. Treatment should occur as early as possible in order to be the most effective.

Materials and methods

The present experiment was carried out on a herd of sheep and goats belonging to a household, from the village of Comoșteni, Dolj County, Romania.

In the present experiment it was formed and studied a group of 10 clinically healthy ewes, during the period August 2017 – April 2018 (approx. 9 months).

The main selection criteria were represented by: the maintenance status, the health status, the age and the number of previous calves. Thus, the group consisted of ewes with: an adequate maintenance status; approximately equal size; clinically healthy and without history of pathologies of any kind; about 3 years old; multiparous, without twin foetuses or a history of foetal dystocia.

The group of 10 ewes were marked and followed during 3 stages of the physiological status, respectively: non-pregnant, pregnant and lactating on day 20, day 40, respectively day 60.

In order to determine the electrolytic parameters, a fraction of plasma obtained by centrifugation, was collected in 2 ml sterile syringes, refrigerated and sent to the laboratory.

Results and discussions

The values of the serum calcium (mg/dL) for each of the ten subjects in the studied group of ewes, in all five physiological stages, are presented in Table 1.

Table 1

Evolution of the serum calcium values (mg/dL) in the studied group of ewes, depending on the physiological status

Physiological status / Subject	Non-pregnant	Pregnant	Lactating		
			20th day	40th day	60th day
1.	10.02	10.43	11.12	10.27	11.29
2.	9.48	8.97	8.68	8.24	9.85
3.	8.11	8.67	8.80	8.11	8.96
4.	10.13	11.08	10.34	9.17	10.94
5.	9.43	9.10	9.15	8.50	9.87
6.	8.50	8.27	8.06	8.19	9.75
7.	8.96	8.07	8.13	7.83	-
8.	9.33	9.08	8.28	8.16	9.68
9.	9.49	9.26	9.11	9.01	8.83
10.	11.05	11.17	10.33	10.02	11.01
$X \pm s_x$	**9.45±2.72**	**9.41±2.68**	**9.20±2.56**	**8.75±2.11**	**10.02±1.34**

In non-pregnant ewes, the mean value of calcium for the group was 9.45±2.72, with a minimum value of 8.11 and a maximum value of 11.05. For the advanced gestation physiological stage, a slightly decrease of the mean value was observed, this being 9.41±2.68, the minimum and maximum values registered in

this period were 8.07 and respectively 11.17. The decreasing tendency of the serum calcium mean value, 9.20±2.56, was also maintained during the early lactation stage on day 20, the minimum and maximum values during this period being 8.06 and 11.12. The lowest mean value was registered on day 40 of lactation, 8.75±2.11, minimum and maximum values of this period of 7.83 and respectively 10.27 being observed. On day 60, the mean serum calcium value increased, reaching the value of 10.02±1.34, with a minimum value of 8.83 and a maximum value of 11.29 during this period.

Therefore, the mean values of the blood calcium concentration in correlation with the physiological status ($X \pm s_x$) are presented in a synthetic manner in Table 2 and Figure 1.

Table 2

The mean values of the blood calcium concentration in correlation with the physiological status ($X \pm s_x$)

Physiological status / Parameter	Non-pregnant	Pregnant	Lactating			Reference range
			20th day	40th day	60th day	
Ca (mg/dL)	9.45±2.72*	9.41±2.68*	9.20±2.56*	8.75±2.11**	10.02±1.34*	9.3 – 11.7

*p > 0.05 - statistically non-significant differences
**p < 0.05 – statistically significant differences

As observed, during this study, important variations of the average values of the calcium concentration were observed, these results being, most likely, associated with the high demands of the foetus, which present an intensive growth rate in the advanced gestation and, at the same time, due to the increased milk synthesis in the early stages of the lactation period.

A statistically significant decrease ($p<0.05$) of the calcium mean value compared with the mean values obtained in non-pregnant ewes, was observed on the 40th day of lactation and, later, an increase of the serum calcium mean value could be observed on day 60 of lactation, with no statistically significant differences ($p>0.05$).

It can also be observed a decrease of the mean values of calcium in pregnant and lactating ewes on day 20, but with no statistically significant differences ($p>0.05$).

Decreased blood calcium concentration may be caused by feeding animals with wheat bran when the animal diet is restricted in order to prevent rumen gasogenesis, as it was often mentioned in the speciality literature (11). However, other authors suggested that this decrease of blood calcium concentration in ewes after parturition and at the beginning of lactation period may be associated with increased calcium secretion through milk and its rearrangement in bones (12).

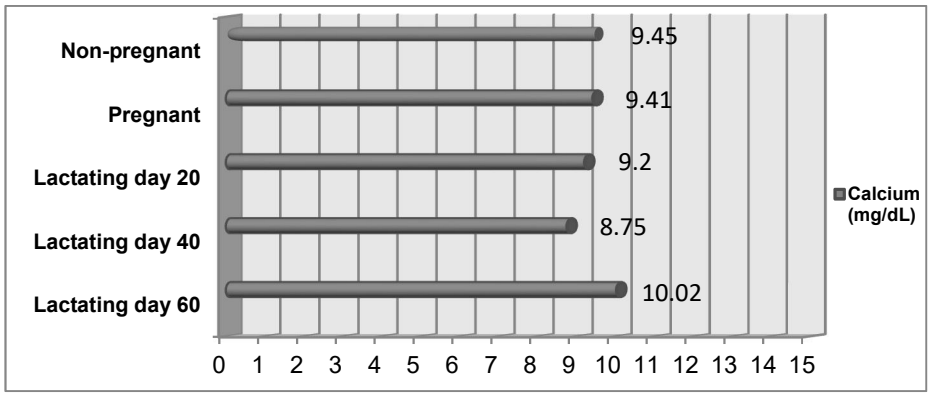

Fig. 1. The dynamics of the mean concentration of blood Calcium evolution in direct correlation with the physiological status in the studied group of ewes

Other authors have also reached similar conclusions (4). Furthermore, calcium and phosphorus are mobilized from bones in similar ways, but much more calcium from the body and blood is removed through milk. Phosphorus is also secreted through milk, but not in the same amount and, consequently, its concentration in blood is much higher than that of calcium, therefore calcium remaining the electrolytic parameter with the most significant variations during late gestation and early lactation periods.

It is well known the fact that the highest demand for calcium is expected approximately 3 weeks prior parturition due to calcification of the foetal skeleton. However, in our study, the lowest mean value of the blood calcium concentration was recorded in the 40th day of lactation and not in late gestation or in the 20th day of lactation as expected, the calcium level increasing and returning to normal in the 60th day of lactation. These findings suggest the fact that the high demand calcium period can be prolonged until the 6th week after parturition. Parturient paresis is a pathological entity that is known to possibly occur at any time from 6 weeks before to 10 weeks after parturition, our study showing a high susceptibly for this disease to occur between 20th and 40th day of lactation, respectively 3rd and 6th weeks after parturition.

Conclusions

The influence of the physiological status on the blood calcium concentration was manifested significantly by important variations of this parameter in both late gestation and early lactation.

During the study, the mean value of calcium decreased non-significantly ($p>0.05$) in late gestation and 20th day of lactation, and significantly ($p<0.05$) in the 40th day of lactation along with the intensification of the milk production.

In both 20th and 40th days of lactation the mean values of the calcium concentration were below the lower limit of the reference range of this species, the lowest value being recorded on the 40th day of lactation, being almost 5% lower than the down limit of the reference range for this species.

These variations can be associated with the high foetal demands during advanced gestation and also with a very high synthesis of milk in the first third of the lactation period.

This study raise awareness on the importance of monitoring the calcium levels and health status of the ewes during lactation, as the lowest value of the calcium concentration was recorded in the 40th day of lactation. Therefore, our study shows that ewes' remain susceptible to develop severe hypocalcaemia during the first 60 days of the lactation period.

Acknowledgement

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BIOCHEMICAL EVALUATION – AN IMPORTANT ISSUE IN PANCREATITIS DIAGNOSIS

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Summary

The pancreas is a heterocrine gland of the digestive tract that has a dual function: exocrine and endocrine. The endocrine role is characterized by the secretion of insulin and glucagon (hormones important in carbohydrate metabolism), but also somatostatin and other pancreatic polypeptides. On the other hand, the exocrine role is manifested by the secretion of pancreatic juice. The exocrine pancreas produces enzymes that are indispensable in the digestion process. If these enzymes are activated for various reasons or the pancreatic juice spill is blocked towards the duodenum, the pancreas begins to digest its own glandular tissue, leading to inflammatory processes and pancreatitis. Excess lipids in the diet, obesity, and hyperlipidemia can be causes of inflammation of the pancreas. The importance of biochemical evaluation in the detection of pancreatitis is underlined clearly by presenting in this research paper two case studies. Both cases showed the evolution of pancreatitis in cats, more specifically in two cats of European breed. They were brought to the clinic with completely different symptoms, which were not enough to establish a complete diagnosis. With the help of biochemical analyses, it was found that both patients suffered from the same disease, despite the distinct symptoms. Moreover, without the clarifications conferred by the biochemical analyses, the possibility of correctly diagnosing a patient decreases significantly. Thus, with the results of the blood analyses, the increased level of lipase was observed and this was subsequently followed by a pancreatitis test which determines the presumed diagnosis. The evaluation by biochemical tests is therefore also necessary in the diagnosis of pancreatitis.

Keywords: pancreatitis, cats, biochemical tests

Pancreatitis is the most common exocrine pancreatic disease in cats. Depending on the levels of pancreatic cell damage and how permanent the affliction is, pancreatitis can be acute or chronic and it can range from mild and asymptomatic to severe (1, 7, 8).

Richard Goldstein – DVM, an associate professor of small animal medicine at Cornell University's College of Veterinary Medicine explains that the digestive enzymes that are in the pancreas normally "sequestered in tiny droplets" that keeps the pancreatic tissue from being in direct contact with the enzymes (15, 16). The specific enzymes are programmed to remain "inactive" until after they have passed through the pancreatic duct into the small intestine. If these digestive enzymes would be active inside the pancreas, they could use the energetic

nutrients (carbohydrates, proteins, and lipids) for nonreversible catabolic reactions – that would digest the pancreas itself (13, 14).

According to many international studies, most cases of pancreatitis are idiopathic. Although the disease is known as arise spontaneously, there are several risk factors that need to be taken into consideration, such as severe blunt trauma, mostly during car accidents, high-rise syndromes, and the ingestion of some drugs (such as phenobarbital, estrogen, thiazide diuretics, and cholinesterase inhibitors) that are likely to play a role in the inflammation of the pancreas. Other important factors in the development of the pancreatic disease are: a diet with an excess of fats, inflammatory bowel disease, obesity and high concentration of lipids in the blood circulation (3, 9, 14).

A study of 157 hospitalized cats diagnosed with pancreatitis in a tertiary care center underlined a few potential prognosis factors that were previously unreported, such as azotemia, parenteral nutrition, hypoglycemia and even withholding antibacterial treatment (6).

Our research paper points the importance of the biochemical evaluation in the detection of pancreatitis which was clearly outlined by the development of two medical cases. Both cases showed the evolution of the pancreatic inflammation in European cats. The research was performed to evaluate the need for biochemical tests in a correct diagnosis of pancreatitis and was motivated by the lack of specific clinical signs that this disease showed.

Materials and methods

Blood samples were taken from two European breed cats. The primary laboratory works consisted of a simple blood panel. Depending on the medical case, the analysis showed elevated levels of globulins, lipase, alanine aminotransferase, and alkaline phosphatase.

After performing the blood analysis we proceeded to do a pancreatitis test – more specifically – a SNAP fPl test (feline pancreas – specific lipase test). The blood samples were placed in a centrifuge and each blood serum was analyzed of the pancreatic test. In both medical cases, the test came out positive, which concluded our pancreatitis diagnosis.

Results and discussions

Pancreatitis is a frequent finding in cats. Because of its variety of symptoms it can be easily misdiagnosed or missed (1). The blood analysis is the most concrete way of an early pancreatitis diagnosis (2, 4, 5). The two medical cases show exactly the importance of these tests.

The first patient was a 6 year old female cat, European breed, sterilized, lived only indoor, was vaccinated up to date and ate only premium food. All of these information are very important in following the development of the disease.

The cat was brought to the clinic in October 2019 with symptoms like sensibility in the throat, diarrhea in form of lax stool and refused the ingestion of any type of food. The following 3 days she received a treatment consisted of antispastic, anti-inflammatory steroids, gastric protection and a complex of vitamins. In the 4th day blood tests were performed (Fig. 1).

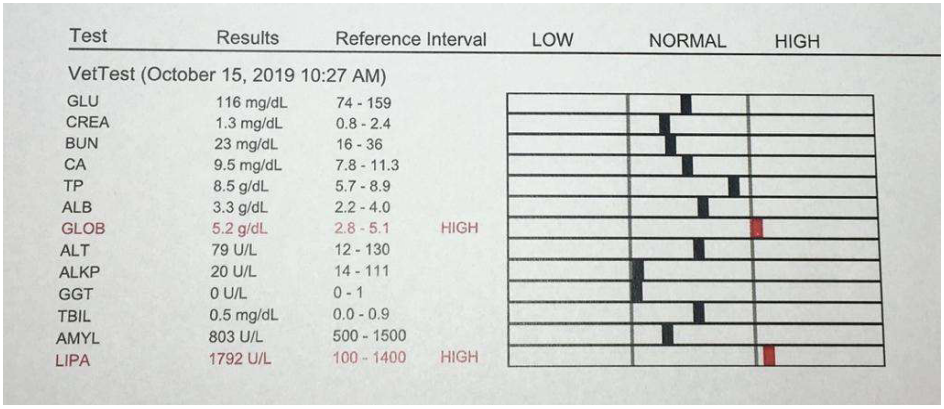

Fig. 1. Blood tests of the first patient (original image)

The elevated globulins (GLOB) levels may indicate a sign of infection, inflammatory disease or immune disorders. However, abnormal results may be due to certain medications, dehydration, or other factors (10, 11, 12). On the other hand, the high lipase (LIPA) levels are known to be an indicator of a pancreatic inflammation. With that taken into consideration, the following step was performing a pancreatitis test, which came out abnormal (Fig. 2). The diagnosis of pancreatic inflammatory disease is only now concrete.

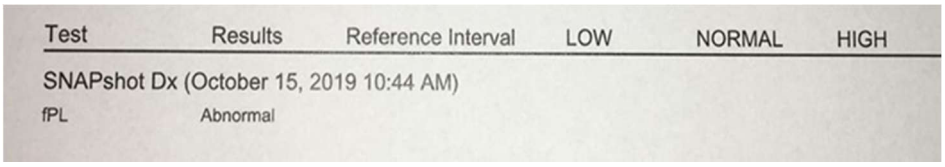

Fig. 2. The SNAP fPl test – feline pancreas, specific lipase test -
of the first patient (original image)

A special diet that consisted of dry and wet food, specific for gastrointestinal support, was given for home care. If it was necessary, the patient was allowed to receive Oralade – a nutritional isotonic drink for oral rehydration and nutrition to aid recovery from vomiting and diarrhea and other gastro intestinal

conditions, where the gut is under stress. In the following 3 days the patient was called to the clinic to receive the aforementioned treatment.

The following day, the cat began to show signs of serious pain and hyper salivation, fact that determined an addition in the treatment of opioid drugs. After about 2 days the patient started vomiting partially digested food or sometimes even a white or light yellow foam. The next 3 days she came to the clinic twice a day: mornings and evenings. In the mornings were administered some antivomitives, vitamins and gastric protection and at evenings only vitamins and gastric protection. The antiemetic medicines had a 24 hour effect. The following days the patient started to eat alone small quantities of wet food. The antivomitives were removed from her treatment and after several days the treatment was stopped altogether.

The patients' symptoms disappeared together with the disease and the cat was in a very good health state at the medical-check – one month since was diagnosed.

The second patient was also a 6 year old female cat of European breed, which had the same living conditions as the first patient. She was sterilized, vaccinated, lived only indoor and ate premium food only. The owners brought her to the clinic for a routine checkup, because of the large quantity of the food she ate. Although the food intake was very high, she showed no signs of gaining weight; more than that, she was even losing weight. Blood tests were performed and the results were the following presented in the following figure (Fig. 3).

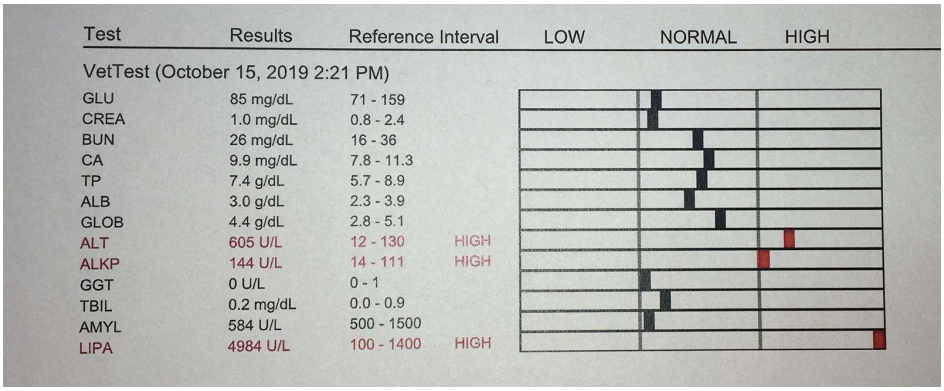

Fig. 3. The blood analysis of the second patient

The routine blood tests showed elevated parameters of alanine aminotransferase (ALT), alkaline phosphatase (ALKP) and high levels of lipase (LIPA). The fact that the ALT levels are high shows sign of possible liver medical problems. High ALKP levels can also show liver problems, a possible manifestation of the Cushing syndrome or in the case of young animals – a sign of bone

development or growth (7, 14). As well, the abnormally elevated lipase parameters are usually common in pancreatitis, diagnosis that was confirmed by performing a pancreatitis test – SNAP fPl test (Fig. 4).

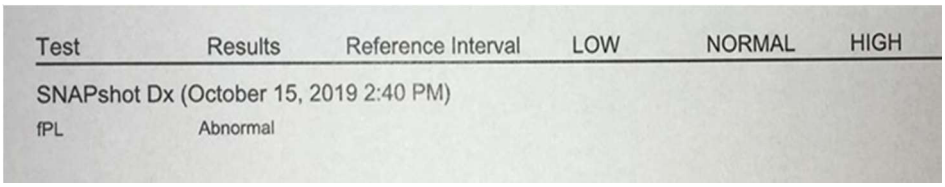

Fig. 4. The SNAP fPl test – feline pancreas- specific lipase test – of the second patient (original image)

It is imperative to notice the lack of symptoms in the case of the second patient. Only with the help of biochemical evaluations can we reach a concrete and certain diagnosis.

Because the cat showed no clinical signs of the disease and was not feeling any pain or discomfort, she was administered some vitamins and diet dry food, special for the support of the digestive system. With time, there was a high chance of a full recovery if the treatment was properly followed.

Conclusions

The pancreatic inflammatory disease is an affection that manifests as a large variety of symptoms. Without the accuracy of a biochemical tests, the symptoms can be attributed to other diseases such as gastroenteritis, ulcer, urogenital and hepatobiliary affections or can even be a sign of the ingestion of a foreign object.

The biochemical tests present a very important role in the administration of a proper treatment, which needs to be followed perfectly, respecting every step.

A correct diagnosis helps the veterinary to establish a right course of treatment adequate for a recovery avoiding medical complications.

Acknowledgement

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MORPHOLOGY OF THE LUMBO-SACRAL PLEXUS IN JACKAL (CANIS AUREUS)

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Summary

The nerves of the lumbosacral plexus raise intriguing questions about their courses, function, and distribution. Lumbar plexus in jackal consist of the seven nerves, while sacral plexus of five. The iliohypogastric nerve is divided in cranial and caudal branches. The nerves are skewed and innervate the same territory as in domestic animals. Close to the greater ischiatic notch the ischiatic nerve is divided into tibial and common fibular nerves. Tibial nerve gives off a tick branch for the biceps femoris, semimembranosus, semitendinous and adductor of the tight. A branch of the common fibular nerve is distributed to the caudal abductor of the crus. Two cadavers from hunted jackals were dissected. Knowledge of the nerves course may allow for safer nerves blockade.

Keywords: lumbo-sacral plexus, nerves, *Canis aureus*

Similarities are evidenced in the comparative topographical anatomy during their evolution although were identified several differences (6).

In dogs description of the ultrasound-guided technique to the transversus abdominis plane (TAP) block and evaluation of the spread of local anesthesia were made (4, 5).

Ultrasound-guided needle insertion is an accurate method for depositing local anesthetic for brachial and lumbar plexus, femoral, and sciatic nerve blocks (1).

Canis aureus is a member of the family *Canidae* that is native to Southeast Europe, Southwest Asia, and Africa. Compared to the wolf, the jackal is smaller and intermediate between fox and wolf. The wolf-like canids group includes the golden jackal, the black-backed jackal, and the side-stripped jackal (3).

Some studies have described the anatomy of the *Canis aureus* with regard of superficial and deep lymph centers (3, 7, 8, 10), the description of the skull and oral cavities (3, 9).

In this context, the comparative anatomical description of the origin of the ventral rami of lumbosacral spinal nerves in *C. aureus* provides information for the understanding of morphological and physiological characteristics that will allow its surgical or medical treatment, as these animals are easy targets of accidents (6).

Materials and methods

The study was conducted on two golden jackals, one male, and one female. The animals originated from the hunting grounds of the Otelec, Timis County, were donated by a private hunter, following the hijacking campaigns.

The specimens used were fresh, and the dissections emphasized four pelvic limbs and two abdominal cavities.

Initially, the incision started from the manubrium sterni to the pecten of the pubis and the skin and connective tissue removed.

To identify the nerves of the lumbar plexus and partially those of the sacral plexus it was necessary to remove the abdominal organs and additional dissections were performed intraabdominal. To highlight the nerves of the sacral plexus the muscles of the pelvic limbs were dissected both superficially and deeply. Nerve tissue samples for histological preparations were performed.

The nerves of lumbosacral plexus have been studied, photographed and described according to the anatomical nomenclature (2).

Results and discussions

The cranial iliohypogastric nerve (Fig. 1) is formed by the ventral branch of L1. It extends between the iliocostal bundles of the quadratum lomborum running caudo-ventraly between the endothoracic fascia and muscles. Consist of two branches, medial and lateral, the latter is longer and supply the muscles and skin of the cranial region of the abdomen.

The caudal iliohypogastric nerve, branch of L2, passes between the iliocostal bundles of the quadratum lumborum muscle, parallel with the previous nerve. Presents two main branches, one medial and one lateral which innervate the muscles and skin of the cranial region of the abdomen. It also consists of a lateral ventral cutaneous branch which supplies the internal abdominal oblique and external abdominal oblique muscles and skin of the cranial abdominal region (Fig. 1).

The ilioinguinal nerve result as the ventral branch of L3. The nerve passes between the iliocostal bundles of the quadratum lumborum muscle and psoas minor muscle close to the end of the psoas major muscle. The two branches of the nerve innervate the muscles and skin of the ventral abdominal region and the cranio-lateral surfaces of the thigh (Fig. 1).

The cutaneous lateral femoral nerve arises from the ventral branches of L3 and L4. The nerve leaves the muscular space between the psoas major and quadratum lumborum muscles and gives off a branch that joins the genito-femoral nerve.

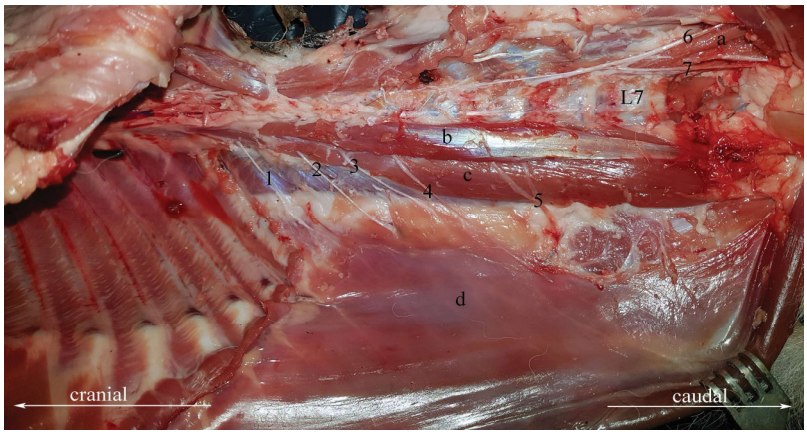

Fig. 1. The nerves of the lumbar plexus, superficial dissection
1. Costoabdominal n.; 2. Cranial iliohypogastric n.; 3. Caudal iliohypogastric n.; 4. Ilioinquinal n.; 5. Genitofemoral n.; 6. Femoral n.; 7. Obturator n.; a. Iliac m.; b Psoas major m.; c. Quadratum lomborum m.; d. Abdominal transvers m.; L7 – last lumbar vertebra

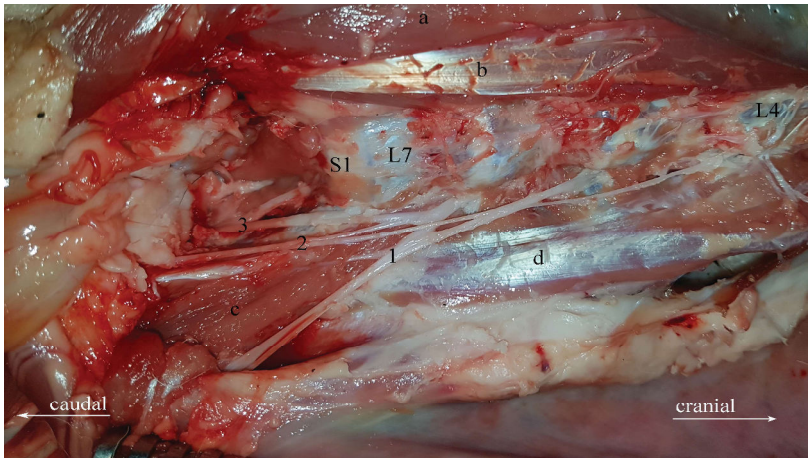

Fig. 2. The nerves of the lumbo-sacral plexus, deep dissection
1. Femoral n.; 2. Obturator n.; 3. Ischiatic n.; a. Quadratum lomborum m.; b. Psoas major m.; c. Iliac m.; d. Psoas minor m.; L 4 forth lumbar vertebra; L7 – last lumbar vert.; S1 – first sacral vertebra

The main nerve innervates the cranio-lateral aspect of the thigh to the coxal tuberosity and greater trochanter.

The genitofemoral nerve detaches from the ventral branches of L4 și L5 nerves and run through the space between the greater psoas and quadratum lumborum muscles. The nerve is single, not divided and long.

The femoral nerve arises from the L4, L5 and L6 within the substance of the iliopsoas and psoas major muscles and. Run ventrally to the lacuna vasorum and leaves the abdominal cavity to join the femoral triangle whereas detach branches for the quadriceps femoris. No cutaneous branches or for the hip joint arise from this nerve (Fig. 2, 3).

The saphenous detaches at the distal third of the femoral triangle, running parallel with the caudal part of the sartorius (Fig. 3).

The obturator nerve arises from the L5 and L6 and is formed within the iliopsoas and psoas major muscles, run parallel with the bodies of the last lumbar and first sacral vertebrae. The nerve leaves the pelvis by passing the obturator foramen and innervates to gracilis, pectineal and adductor muscles (Fig. 4).

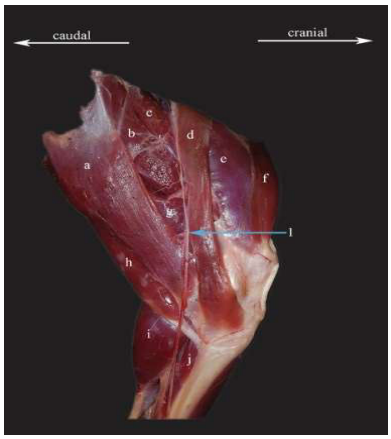

Fig. 3. Superficial dissection of medial aspect of the thigh
1. Saphenous nerve; a. Gracilis m.; b. Adductor m.; c. Pectineal m.; d. Sartorius, caudal part; e. Vastus medialis; f. Sartorius, cranial part; g. Semimembranous m.; h. semitendinous m.; i. Medial gastrocnemian m.; j. Popliteal m.

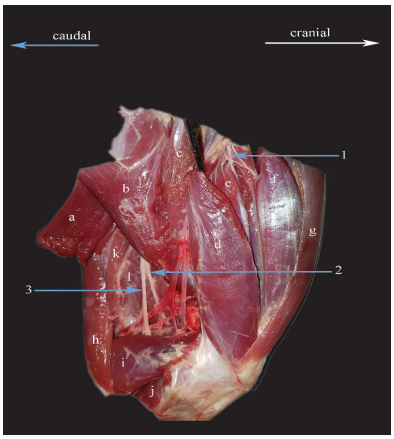

Fig. 4. Medial aspect of the thigh
1. Femoral n.; 2. Common fibular n.; 3. Tibial n.; a. Semimembranous; b. Adductor m.; c. Pectineal m.; d. Medial vastus; e. Vastus intermedius; f. Rectus femoris; g. Caudal part of sartorius m.; h. Semitendinous m.; k. Caudal abductor of the crus; i. Medial astrocnemian m.; j. Popliteal m; l. Biceps femoris m.

The cranial gluteal nerve arises from the ventral roots of the L6, L7, and S1 and leaves the pelvic cavity through the greater ischiatic foramen to innervate the

rump musculature. Innervates the deep and middle gluteal muscles and tensor of the fascia lata.

The ischiatic nerve is the largest nerve of the plexus, formed by the ventral roots of L6, L7, S1 and S2. After passing the greater ischiatic foramen, near the ischiatic spine the nerve divides in fibular and tibial nerves (Fig. 4, Fig. 5).

Common fibular nerve has an almost vertical direction, passing the intermuscular space between biceps femoris, deep gluteal, gemelli, and adductor muscles. Close to the hip joint from the parent nerve detaches lateral surae cutaneous nerve whose branches, proximal and distal innervate the caudal abductor of the thigh and the skin from the latero-caudal aspect of the thigh.

Lateral to head of fibula the main nerve divides in the superficial and deep fibular nerves.

The superficial fibular nerve leaves the parent nerve dorsal to the hip joint and extends distally between the distal part of the peroneus longus muscle and long digital extensor where divides into the superficial and deep branches (Fig. 5).

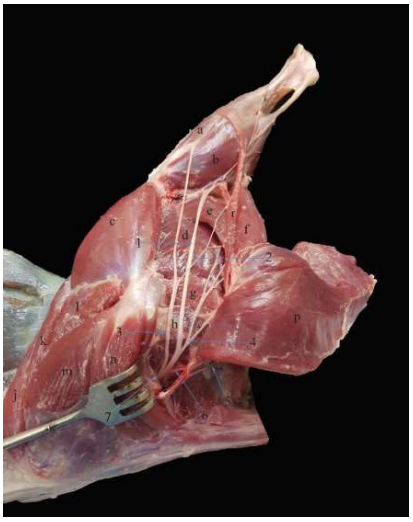

Fig. 5. Caudo-lateral aspect of the thigh
1.Tibial n.; 2. Lateral cutaneous sural n.; 3. Common fibular n.; 4. Muscular branches; 5. Pudendal n.; 6. Femoral caudal cutaneous n.; 7. Ischiadic n.; a. Cranial tibial m.; b. Lateral gastrocnemian m.; c. Lateral vastus; d. Adductor m.; e. Semimebranous m.; f. Semitendinous m.; g. Quadratus m.; h. Gemelli mm.; i. Internal obturator m.; j. Cranial part of sartorius; k. Tensor of fascia lata; l. Cranial part of superficial gluteal m.; m. Midle gluteal m.; n. Caudal part of superficial gluteal m.; 0. Coccigeal m.; p. Biceps femoris m.; r. Caudal abductor of the crus

The *tibial nerve* is larger and separates from the common fibular nerve close to the caudal aspect of the hip joint. In the middle third of the caudal aspect of the stifle crosses the lateral cutaneos surae branch (Fig. 5).

Conclusions

Cranial iliohypogastric n. run parallel with the costoabdominal n. and the caudal iliohypogastric n. follows the previous nerve.

Genitofemoral n. results from the ventral branches of L4 and L5 nerves.

Ischiatic n. is formed by many branches of L7, S1, S2. A few cm bellow the hip joit divides in the common fibular and tibial nerves.

Caudo-ventrally to the hip joint, tibial n. gives many muscular branches.

Proximo-caudal to the femoral condyles, tibial n. gives the caudal cutaneous sural n.

Caudal to the knee joint, the tibial nerve gives muscular branches for triceps sural m.

Caudal to the hip joint, common fibular n. gives the lateral cutaneous sural n.

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THREE-DIMENSIONAL (3D AND 4D) ULTRASONOGRAPHY IN VETERINARY OBSTETRICS AND GYNECOLOGY

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Summary

The most widespread imaging technique that allows the investigation of soft biological structures is ultrasonography. Among the many applications of ultrasonography, are highlighted medical procedures on skin and musculoskeletal tissues, cardiovascular, urinary and reproductive systems. Ultrasonography has been the instrument of medical research since World War II. In 1942 the first paper in the field of medical ultrasonography was published. From the 1970s when ultrasound technology began to be marketed, to this day, technology has been continuously improved. The purpose of this paper is to review data related to three-dimensional ultrasound 3D and 4D technology connection to veterinary medical practice. Aspects such as: historical evidence, evolution and current status of 3D and 4D technologies, the benefits and inconveniences, the risks, the perspectives for use in clinical veterinary practice, especially in the obstetrics and gynecology field, are presented.
Keywords: 3D and 4D ultrasonography, veterinary obstetrics and gynecology

Among the imaging techniques of examination, the most used in the field of Obstetrics and Gynecology, as well as for the examination of other organs and soft tissues, is ultrasonography. There are several advantages associated with ultrasonography: it is a non-invasive technique, the accuracy of the diagnosis is high, the equipment is easy to use, the portable devices are available and the costs are lower compared to other imaging techniques. Generally, the evaluation of the morphology and pathology of the organs is performed by the conventional two-dimensional 2D ultrasonography. Additionally to 2D ultrasonography that allows evaluation imaging of single planes, three-dimensional 3D/4D ultrasonography provides more information about the third dimension of the investigated area through options for viewing and analyzing volume data. In the three-dimensional 3D ultrasonography, the data obtained from 2D images are reconstructed into a still 3D image, using specialized software. Real-time display of the 3D data, during dynamic scanning, is referred to as 4D ultrasonography.

Historical evidence

Ultrasonography has been the instrument of medical research since World War II. In 1942 the first paper in the field of medical ultrasonography was published. In 1987 the Center for Emerging Cardiovascular Technologies at Duke University headed by Olaf von Ramm begins to develop a real-time 3D volumetric scanner for imaging the cardiac structures (26).

Baba K. wrote a comprehensive book on diagnostic ultrasound in obstetrics and gynecology including 3D ultrasound in 1992 (2) and edited together with Jurkovic D., Three-dimensional ultrasound in obstetrics and gynecology, the first book devoted entirely to 3D ultrasound, in 1997 (4).

The technique of ultrasonography has continued to develop, so in 1996 Takashi Okai did first research on 4D fetal echocardiography (3).

With the twentieth century, some veterinary researchers have begun to publish the results obtained by three-dimensional ultrasonography such as the technique used to study the mammary gland in cattle (12), pregnancy in the elephant (10), in dogs and cats (1, 5, 8, 15, 19), in horses (20), in African lion (16), in goats (21).

The evolution and current status of 3D and 4D technologies

Conventional ultrasonography 2D is an imaging technique that obtains the image of a section through an organ. In order to facilitate the investigation of the morphology of the organs, the diagnosis and prognosis of some pathologies, it is necessary the three-dimensional visualization that offers an image of the volume of the organ. In the 3D visualization, the organs appear as in a photograph, and to remove the artifacts during the respiratory movements of the mother, cardiac shock or involuntary movements, 4D ultrasonography can be used, which is a real-time 3D technique. For three-dimensional real-time visualization, three stages have been completed: acquisition, reconstruction and visualization. Huang and Zeng (17), propose, for a good 3D visualization, the following data acquisition techniques: 2D array transducers, mechanical localizers, mechanical 3D probes (a regular linear array transducer is motored to rotate, tilt, or translate with in the probe under the computer control; multiple 2D images are acquired over the examined area when the motor is activated. The axis of rotation, tilt, or translation can be used as a reference frame for 3D image reconstruction) and freehand scanners. Reconstruction takes place after three types of algorithms: Voxel Based Methods (VBMs), Pixel-Based Methods (PBMs) and Function-Based Methods (FBMs) (17).

The benefits and inconveniences

Benefits: in the investigations of gynecology and obstetrics, three-dimensional ultrasonography is a much more sensitive method than two-dimensional ultrasonography, because it allows visualizing the morphological and pathological structures both in the visualization plan and in the inclined plane (11, 13, 14). Thus the three-dimensional technique allows visualization of superficial fetal defects, cracks of the face, defects of the secondary palate, capture of a volume of the fetal heart. In addition to the advantages listed, vascularization and blood flow can be quantified volumetrically and real-time three-dimensional ultrasonography perceives fetal movements, suggesting fetal viability (7).

Inconveniences: among the reasons why 3D ultrasonography is less used than the two-dimensional method in veterinary medicine, may be mentioned: higher costs of equipment, lack of specialists to use this technique, software and hardware settings are created for use in human medicine and not are adapted to veterinary anatomy (15). Compared to human medicine, in veterinary medicine, obtaining three-dimensional ultrasonographic images is more difficult, as 3D images obtained may be of poor quality due to artifacts that appear during respiratory movements or agitated animals during examination, which may require anesthesia.

Risks

For a safe ultrasound examination the ALARA concept ("As Low As Reasonably Achievable") must be respected.

The potential risk to which the patient is exposed during an ultrasonographic examination is the energy potential of the ultrasound to generate bioeffects through cavitation and harmful phenomena. The potential to generate the tissue cavitation phenomenon is indicated by the mechanical index (IM), but in mammalian fetuses this phenomenon has not been demonstrated, due to the lack of a gas-fluid interface (25). In contrast, the harmful potential along the ultrasound beam has been experimentally demonstrated in animals and is expressed by the thermal index (TI). Sheiner et al. (25) aimed to compare the ultrasound-produced bioeffects during conventional ultrasound evaluation of conventional (2D) and three-dimensional (3D and 4D) ultrasonography. They evaluated 40 pregnant women, with the age of pregnancy between 25 and 37 weeks, using three different types of ultrasound. Comparing the two indices, IM and TI, they found that there are no major differences between the evaluations with the three ultrasonography devices of different companies. There were no significant variations even between TI associated with 3D and 4D volume acquisition, compared to conventional ultrasonography. The resulting IM in 3D volume purchases was significantly smaller than the IM in conventional acquisitions and real-time 3D volume acquisitions.

TI and IM depend on the actual exposure time to the ultrasound beam. Pooh et al. (23) evaluated the risks of three-dimensional ultrasonography (3D and 4D) compared to conventional ultrasound and found that the techniques are safe as long as the exposure time is less than half an hour.

The perspectives for use in clinical veterinary practice, especially in the obstetrics and gynecology

Biopsy and standardization of rectal cancer, diagnosis of fetal and gynecological abnormalities, breast examination, are applications of three-dimensional ultrasonography commonly used in human medicine. In veterinary medicine 3D/4D ultrasound is used for the evaluation of gastric disorders in dogs, renal ultrasound and diagnosis of bladder disease (9, 15, 22). In the field of veterinary Obstetrics and Gynecology, three-dimensional ultrasonography is used for the evaluation of fetal evolution, determination of fetal sex, screening of fetal abnormalities, detection of ectopic gestation, prediction of parturition. In Gynecology, 3D ultrasound is a real help in pelvic mass evaluation, fertility management, ovarian cancer screening.

In recent papers, some authors present the use of three-dimensional ultrasonography to study the equine fetus. The determination of equine fetal sex is done by ultrasound technique and is possible by transrectal approach (is more invasive and the acquisition must be made in a short time by an experienced operator) after 2 months after the mount and by transabdominal approach, after three months after the mount. Pricking et al. (24) determined fetal sex in pregnant mares in the second and third gestation period, by transabdominal approach, based on fetal gonadal structures, specific vascularization at the gonad level, and external genital organs. The gonads were identified with conventional ultrasonography, and the details were visualized with three-dimensional technique. If the gonads were located near the bladder it indicates the male fetus, and the gonads located near the kidneys indicate a female fetus. Kotoyori et al. (20) studied the fetus equine in the first half of gestation with the 3D ultrasonography, using the transvaginal and transrectal approach and characterized the fetal development through detailed images of the body surface of the limbs and genital tubercle, during 90-150 days of gestation. Becsek et al. (6) approached the uterus of the mares during early gestation, through transrectal approach and studied fetal volumetry using 3D ultrasonography, obtaining measurements superior to conventional 2D ultrasonography.

Karadaev et al. (18) and Kumar et al. (21) used three-dimensional ultrasonography to study fetal evolution in gestation in goats. Karadaev and his colleagues (18) visualized the internal organs and the individual parts of the fetal goat in the second and third trimester of gestation. Three-dimensional visualization of the head and fetal body was possible from the beginning of the second gestation period (60 days of gestation). The outline of the skull and fetal orbit could be clearly

seen at 90 days of gestation; also in the third trimester of pregnancy the researchers obtained three-dimensional images of the internal organs: kidney, heart, ribs, fetus network. Kumar and colleagues (21), compared conventional ultrasonography technique, 2D, with three-dimensional technique in a study of the uterus and product evolution of conception, starting from day 20 to 120 days of gestation at the Beetal goats. They obtained the first 3D image of the conception product on day 24 of gestation, through transabdominal approach, and in 39 days of gestation, they visualized clear images of the conception product, the amniotic membrane and the umbilicus. The heart, kidneys, liver, bladder and stomach of the fetus were observed at 76 days of gestation. Following this study, the researchers concluded that conventional ultrasonography is more appropriate for fluid visualization, and three-dimensional ultrasonography provides clear images of fetal attachment to the endometrium.

Fasulcov et al. (11), using 3D ultrasonography, examined the udder of lactating cows. They captured detailed images of the anatomical structures: papillary orifices, teat wall and teat cistern, teat canal, mammary parenchyma, rosette of Furstenberg. Thus 3D ultrasonography can assist in the diagnosis and prognosis of pathological changes in the mammary gland. The disadvantage found by the researchers was a delay in the reconstruction of two-dimensional images in three-dimensional images, this problem could be avoided by the use of real-time three-dimensional ultrasonography.

Conclusions

In conclusion, three-dimensional ultrasonography 3D/4D is an imaging technique that comes to complement conventional 2D ultrasonography, with detailed information that can be useful in tracking the evolution of a structure, in establishing a diagnosis and prognosis, both in the field of obstetrics and gynecology, as well as the rest of the veterinary clinical activity.

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IDENTIFICATION OF POTENTIAL PATHOGEN BACTERIA FROM MILK AND DAIRY PRODUCTS

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Summary

Milk and the dairy products that derive from it, are commonly used in human alimentation. Among other dairy species the cow's milk is the most produced and consumed. Because of its high nutritive content, milk can support the development of a rich variety of microorganisms. The microorganisms are playing different roles in milk like: dairy fermentation, health assuring through their probiotic role, but there are also bacteria that produce different types of diseases, like mastitis in cow collective and enteritis at human, mainly through the toxins they produce. Some of these pathogenic bacteria can develop a certain resistance against antibiotics. The current paper aims to identify the possible pathogen bacteria present in milk, using 10 varieties of dairy products collected from different locations. The DNA was isolated and purified from bacterial cells that were found in milk and used as template in PCR experiments in order to detect pathogenic bacterial strains and some of the most common antibiotic resistance genes. Not lastly, the DNA is used to study the prevalence and genotypes of the identified bacteria.
Keywords: pathogenic bacteria, dairy products, virulence genes, antibiotic resistance.

Milk represents a basic food regarded as a highly nutritious and healthy food with great value in human diet (5, 15). But milk it's also an important source of bacterial infection for human when it is consumed raw and unprocessed. Because of its high nutritive content, milk can support the development of a rich variety of microorganisms. The microorganisms are playing different roles in milk like: dairy fermentation, health assuring through their probiotic role, but there are also bacteria that produce different types of diseases, like mastitis in cow collective and enteritis at human, mainly through the toxins they produce (9). The dairy industry demands a high volume of milk in order to maintain the quality and quantity of an ever growing demanding of milk diaries. One of the biggest responsibilities of the milk industry, both big farms and small producers is to ensure a sure and high-quality milk and milk products to the population in order to prevent food borne diseases. The zoonosis and food borne diseases are a priority to in programs assuring the public health, and there are strictly monitored (8). The presence of residual in milk, contaminators and pathogens is an indicator of milk quality. The fresh raw milk and fresh dairy products are a potential disease transmitter. The microbial contamination can come from bacteria identified in grassland or animal carriers of *Salmonella, Mycobacterium bovis, Escherichia coli*. Other sources of bacterial contamination are mastitis and poor hygiene (5, 9). A poor hygiene and cleansing procedure may lead to

contamination of milk. Mastitis is a multi-etiologic disease of the mammary gland characterized mainly by reduction in milk production and milk quality due to intramammary infection by pathogenic bacteria. (11, 13)

The indigenous microbiota from milk is *Staphylococcus spp., Enterococcus spp, and Lactococcus spp*. But some species can be highly dangerous pathogens, as *Staphylococcus aureus*, it has a lot of virulence factors, among which the secreted toxins play an important role, leading to cell death. In particular, S. aureus produces potent hemolysins, factors that inhibit the complement cascade or prevent recognition by host defenses (4, 10, 14).

This study aims to identify the possible pathogen bacterial including *Staphylococcus aureus* in milk and dairy products. Various PCR methods for bacterial identification had developed in the last years like single strand confirmation polymorphism or desaturating gradient gel electrophoresis, methods with great value in monitoring the microbial dynamics. In this study it is been used the DNA sequencing, which is a process of determination the constituency of a unique sequence of nucleotides (adenine, guanine, cytosine, thiamine) form an ADN molecule. It is used to find the initial sequence data from an organism in order to identify it or to compare it with others. It is highly used in molecular biology in order to understand the effects of particular genes and to identify the genetic composition of plasmids or bacteria.

Materials and methods

Biologic material

For the current experiment 10 particular samples were used, 10 varieties of dairy products collected from different locations in Timisoara.

The biological material origin is a following:

Table 1

Biological material used and provenience

Samples	**Provenience**
A, B	Sweet cow cheese – from the local market
C, D	Salted cow cheese – from the local market
E	Milk – from the local market
F	Milk from a local seller
G, H, I, J	Milk – university project – possible mastitis cows

The G, H, I, J material was used from milk samples that were brought from cows with mastitis, and ought to be studied in the "*Bioeconomic approach to antimicrobial agents - use and resistance*" project.

The primers used for this paper were selected from scientific papers and they were synthesised by Eurogenetec, Belgium (Table 2).

Table 2

Primers and identified genes

	Identified genes	Primers sequences
Identification of the pathogenic bacteria	27F	AGAGTTTGATCCTGGCTCAG
	U1492R	GGTTACCTTGTTACGACTT
	aac(6')aph (2")_FW	GAAGTACGCAGAAGAGA
	aac(6')aph (2")_RV	ACATGGCAAGCTCTAGGA

Methods

The DNA isolation from milk samples

The DNA was isolated from sedimented cells from milk with the help of Quick Blue kit using the producer protocol. Before the isolation, the milk cells were purified from the fresh milk samples. 30 ml of milk were equally distributed and centrifuged, then the supernatant was cleared and the remaining cells were ready for DNA isolation. The quantity and quality of the extracted DNA was verified using the spectrophotometry method using the UV-VIS Nanodrop 8000 (Thermo Scientific) spectrophotometer.

The isolated DNA was further purified using the Genomic DNA clean-up kit, following the producer protocol.

Electrophoresis analysis in agarose gel

The electrophoresis it is a method that determines the separation of molecules using their electrical charge. This method depends on the dimension of the DNA fragments, gel concentration, temperature, the electrical tension used for migration. The migrated DNA fragments were afterwards seen in UV light.

Sequencing of DNA fragments

The fragments of DNA used to identify the bacteria were isolated from the agarose gel using the Monarch DNA Gel extraction (New England Biolabs, USA).

The DNA amplicons were sent for sequencing at Macrogen Laboratories, Netherlands, and subjected to *in silico* analysis using the NCBI Database.

Results and discussions

After the isolation and purification of the DNA the samples were verified with the spectrophotometer which measures the number of protein molecules in the given samples but most important the quantity of DNA present in each sample. The results are listed in the Table 3.

The obtained results allowed the further steps to be taken because it was considered that there was enough DNA both qualitative and quantitative, to pursue the enzymatic reaction.

Table 3

DNA sample concentration

Crt.No.	Samples	Concentration
1.	A	4.43 ng/nl
2.	B	3,63 ng/nl
3.	C	17.22 ng/nl
4.	D	3.05 ng/nl
5.	E	7.81 ng/nl
6.	F	17.12 ng/nl
7.	G	16.43 ng/nl
8.	H	1.90 ng/nl
9.	I	21.40 ng/nl
10.	J	9.93 ng/nl

First step of this study was to identify bacterial DNA in the dairy samples. The identification was made by migrating two DNA sequences that are present in bacterial genome, being molecular markers for two bacterial genes, 27 F and U1492R (Fig. 1).

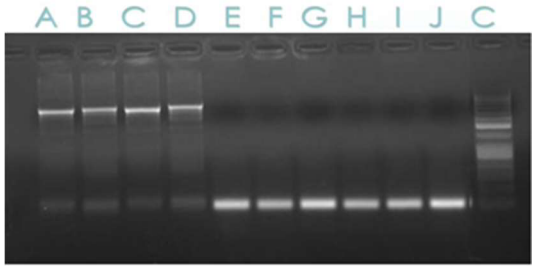

Fig.1. Identification of bacterial DNA in the analyzed samples. Lanes A-J, milk samples considered in this study; C – DNA Ladder, PCR Marker (Promega)

There were positive results in samples A to D, in this samples there can be found bacterial DNA. The A-D fragments were isolated and sent for sequencing in order to find if in the bacterial DNA there was *Staphylococcus aureus* DNA. The results were interpreted by uploading the gene sequence into NCBI database and comparing it with the specific sequence of *Staphylococcus aureus* already uploaded into this database. This analyze revealed a similarity of 74% between the specific sequence and the sequence isolated in this study.

The next step was to identify the existence of an antibiotic resistance gene, aac(6')aph(2") gene which encodes an enzyme that gives Gram positive bacteria resistance to Aminoglycoside antibiotics and disable their function, like gentamicin and neomycin. Gentamicin is currently used in treating mastitis in cows.

After the DNA migration, it is clear to see that samples A B C D F G H and J are positive for this particular gene aac(6')aph(2") which is the gene responsible for the resistance of bacteria to Aminoglycoside antibiotics.

This study was based on finding pathogen bacterial DNA in milk and dairy products, and the resistance genes. With a 74% matching sequence for *S. aureus* and the existence of the resistance gene to Aminoglycoside antibiotics in 7 samples out of 10 the purpose of the current study was achieved (Fig. 2). Further investigation need to be taken in order to see what type of bacteria has the resistance gene.

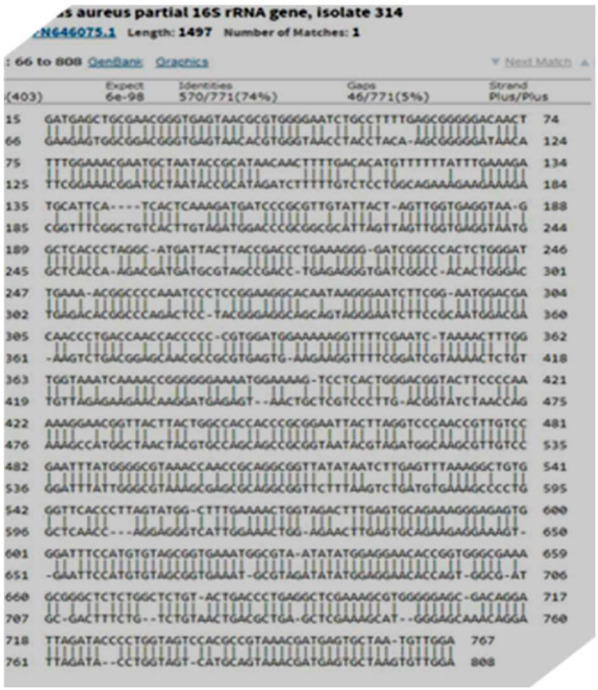

Fig. 2. Alignment of bacterial DNA obtained in this study with the DNA reference gene for *Staphylococcus aureus*, stored in NCBI Database

Since *Staphylococcus aureus* is considered an important pathogen in mastitis in cows and infections in humans. Mastitis in large farms raises an economic problem of the cost of treatment and beside this the milk production is low, the risk for antibiotic resistance is growing. The importance for the public safety of this bacterium it is that is manifested through food borne diseases. (6, 7, 12).

Buckle (6), in 2019, did a monitoring program in Germany on mastitis causes and the data gathered from this program was used to appreciate the herd prevalence of 15 mastitis causing bacteria and lactamase gene in bulk tank milk from 31 farms.

She discovered that from all the samples positive to lactamase gene, only 58% were positive for *Staphylococcus aureus* and 42% for *Staphylococcus sp. Staphylococcus sp.* were the most abundance bacteria found in milk and the presence of lactamase gene was correlated with its existence (1).

Wang (13) in 2018, collected 195 milk samples from 2 farms in Beijing. Of the 195 samples, 90 were found positive for *Staphylococcus aureus*. These were tested for resistance genes and the most common resistance found was at penicillin and ciprofloxacin (3).

Basanisi (5) in 2017, tested over 3760 samples of milk samples and dairy products collected from 2007 to 2014. There were 484 *S. aureus* strains isolated and 40 of them were methicillin resistant (2).

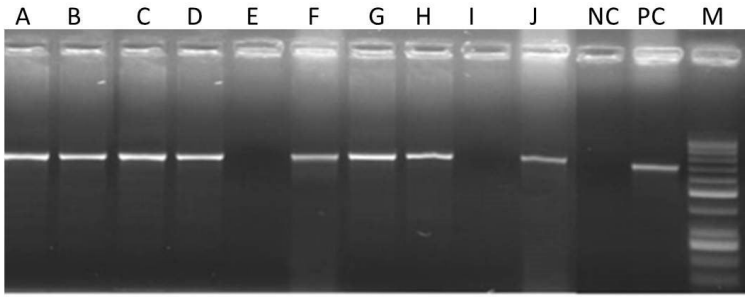

Fig. 3. Migrated DNA for the ten samples and their results for aac(6')aph gene. Lanes A-J, milk samples considered in this study; NC – negativ reaction control; PC – positive reaction control; M - DNA Ladder, PCR Marker (Promega)

Conclusions

Abusing of antibiotics usage, it is a facing and growing problem in veterinary medicine and public health. The bacteria evolve and develop antibiotic resistance genes, as the one that has been identified in the present study. The bacteria might develop a gene for resistance that does not express itself phenotypically but it can send it on to other bacteria in which the manifestation of the gene can become an issue for the health and treatment system.

Acknowledgement

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STUDY OF THE ETIOLOGY OF ACUTE KIDNEY INJURY IN DOGS

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Summary

There are a number of underlying conditions that can lead to AKI, and ultimately to ARF. Unfortunately, despite diagnostic workup, a definitive underlying cause for development of AKI is often not found. Acute kidney injury is characterized by a rapid onset of renal insufficiency/failure, reduction in glomerular filtration rate and renal plasma flow, and the clinical and biochemical aftermath of the excretory failure. Clinical and ultrasound investigations in this study were conducted at the Medical Clinic of the Bucharest Veterinary Medicine Faculty during March 2019 - October 2019 on a number of 10 dogs of different races and ages presented for full evaluation. The ultrasound was performed using the MyLab.30 ultrasound with a convex or linear probe with a frequency of 5-8 MHz. Diseases with AKI included a wide range of pathological processes dominated by acute uremic crisis: 40% of toxic nature, 20% babesiosis (n = 2), 20% urinary infections (n = 2) and post-renal obstruction 20% (n = 2). The etiology of AKI and the type of injury are the most important prognostic factors for affected dogs and their identification is therefore essential for an accurate prognosis.

Keywords: dogs, etiology, acute kidney injury

There are a number of underlying conditions that can lead to AKI, and ultimately to ARF. Unfortunately, despite diagnostic workup, a definitive underlying cause for development of AKI is often not found (4, 5).

Acute kidney injury is characterized by a rapid onset of renal insufficiency/failure, reduction in glomerular filtration rate and renal plasma flow, and the clinical and biochemical aftermath of the excretory failure (1, 2, 6).

It does not appear only as a result of the immediate action of aggressive factors but also as a result: a decompensation of the kidney with parenchymal interest: inflammation / edema tubular and / or interstitial, tubular ischemia, drug allergies, autoimmune diseases, infections, tubular toxico-necrotic disorders: aminoglycosides, ethylene glycol, NSAIDs, heavy metals. The condition can recognize prerenal causes (reduction of renal perfusion, C.I., septic shock, digestive fluid loss), postrenal causes (urethral obstruction, bladder rupture) or any combination of these (7).

As a clinical dominant sign = oligo / anuria (<0.5 ml/kg/hr, respectively <0.1 ml/kg/hr) (3).

Materials and methods

Clinical and ultrasound investigations in this study were conducted at the Medical Clinic of the Bucharest Veterinary Medicine Faculty during March 2019 - October 2019 on a number of 10 dogs of different races and ages presented for full evaluation.

The ultrasound was performed using the MyLab.30 ultrasound with a convex or linear probe with a frequency of 5-8 MHz.

Results and discussions

Diseases with AKI included a wide range of pathological processes dominated by acute uremic crisis: 40% of toxic nature (n=4), 20% babesiosis (n=2), 20% urinary infections (n=2) and post-renal obstruction 20% (n=2).

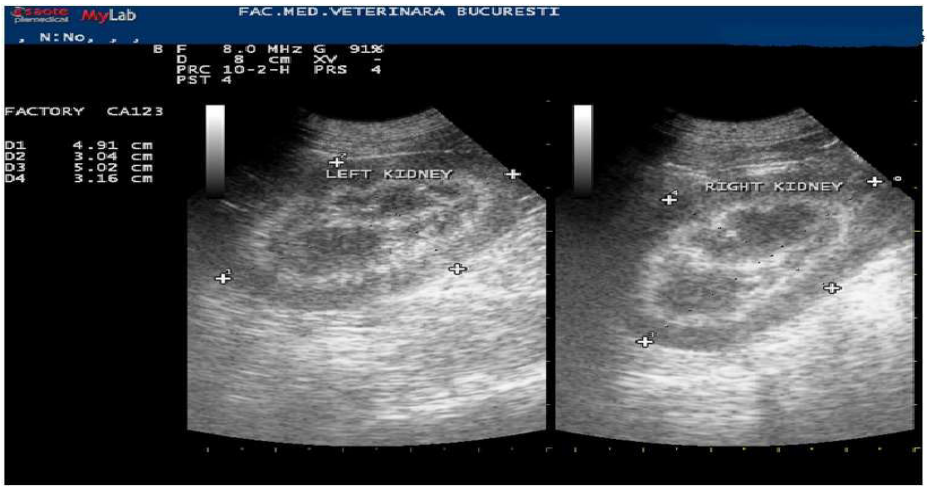

Fig. 1. Right and left kidney longitudinal section. Renal nephrotoxicity with ethylene glycol with preservation of cortico-medullary delimitation. Renal medullary with bilaterally hyperecogenic diffused aspect with significant changes in echostructure and echogenicity.
Diagnosis: Ethylene glycol intoxication with acute oliguric renal failure

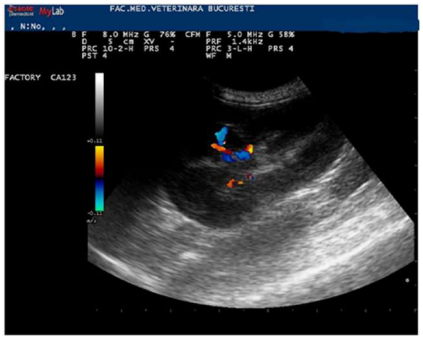

Fig. 2.The right kidney has a congestive aspect, modification of the cortico-medullary ratio 1/1, the renal cortex with a general hypoecogenic aspect in relation to the spleen with the increase of the parenchymal index and the highlighting of the intra-parenchymal vascular formations (arteries and interlobular ectasia).
Diagnosis of: AKI - caused by massive infestation with *Babesia canis*

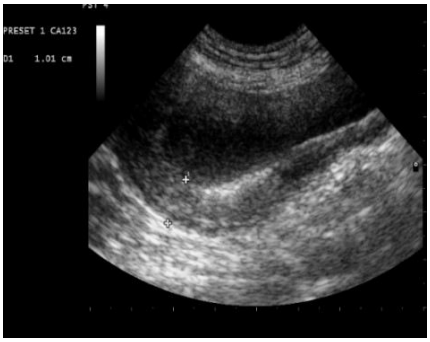

Fig. 3. Urinary bladder with homogeneous thickening of the bladder wall (1 cm). Acute renal failure following severe cystitis
The result of uroculture revealed the presence of an infection with *E.coli*

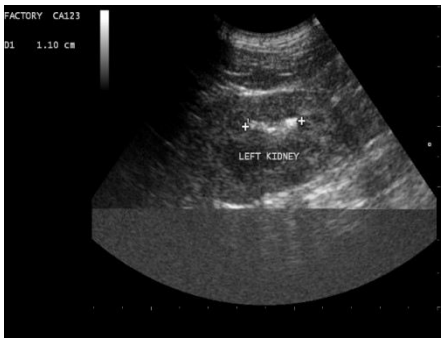

Fig. 4. Left kidney with slightly irregular contour with preservation of characteristic renal architecture. It is observed in the cortico-medullary in the demarcation zone the presence of a single hypercogene (transverse diameter 1.1 cm), accompanied by the posterior shading phenomena, without the distension of the pielocaliceal system.
Diagnosis: Acute renal failure secondary to ureteral obstruction and nephrolithiasis

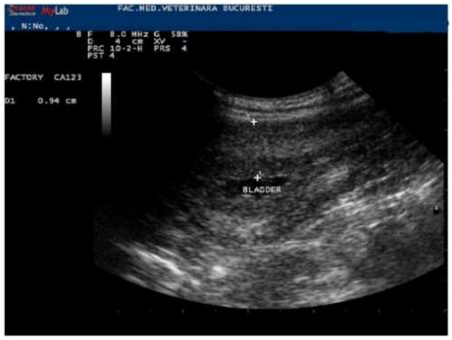

Fig. 5. Irregular contouring bladder with uniformly thickening of the bladder wall with characteristic parietal highlighting (0.94 cm)

The study reveals an equal proportion between the sexes in the aki disorder (Fig. 6) and a percentage distribution by age (Fig. 7) without statistical value being dependent on the etiology of the disease (Fig. 8).

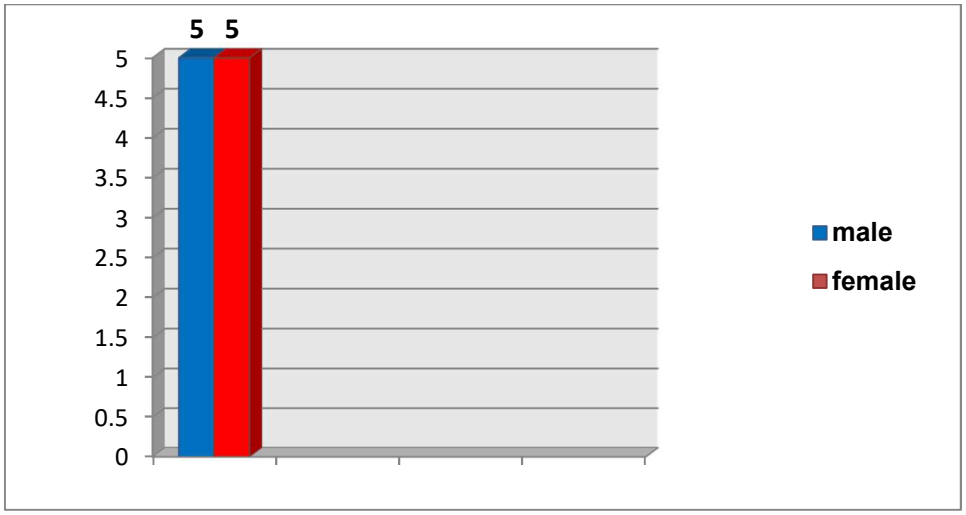

Fig. 6. Gender distribution of patients with AKI

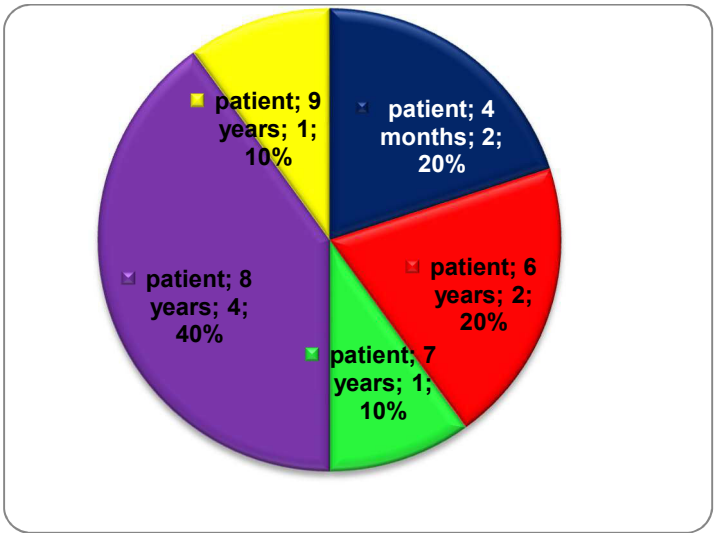

Fig. 7. Percentage distribution by age of patients with AKI studied

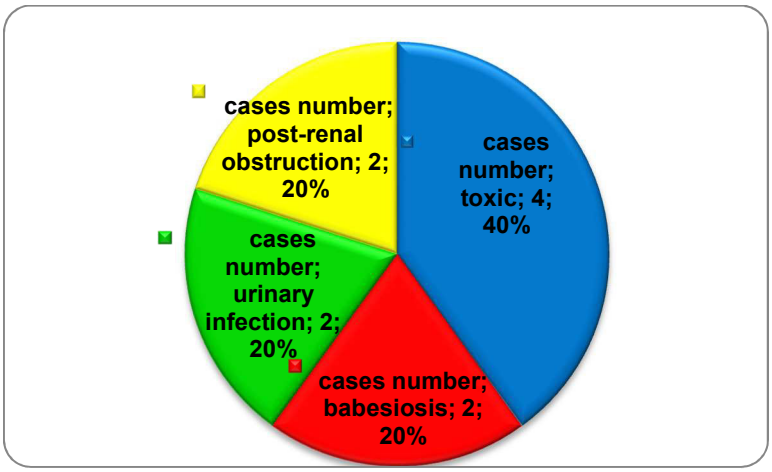

Fig. 8. Percentage distribution of the etiology of AKI in the patients studied

Conclusions

The etiology of AKI and the type of injury are the most important prognostic factors for affected dogs and their identification is therefore essential for an accurate prognosis.

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ECHOCARDIOGRAPHIC EVALUATION IN DOGS WITH DILATED CARDIOMYOPATHY

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Summary

Dilated cardiomyopathy (DCM) is the most common myocardial disorder in canines. It affects mainly large breed dogs but can also be encountered in medium sized breeds such as Cocker Spaniel, and it develops during adulthood. The diagnostic protocol includes physical examination, 24-hour Holter monitoring, thoracic radiography and electrocardiography. The aim of the study is to describe the most specific echocardiographic measurements in dogs with DCM in order to affirm the presence of this disease in the referred patients. Client-owned dogs with DCM were retrospectively selected from the Veterinary Teaching Hospital of the Veterinary Faculty of Iasi between 2016 and 2019, based on history, physical examination, thoracic radiography, electrocardiography and complete electrocardiography. All dogs showed increased values for left ventricular internal diameters (LVID) in systole and diastole, end systolic and diastolic ventricular volumes, E point to septal separation (EPSS) and left atrium to aorta ratio (La/Ao). There was also a decrease in shortening and ejection fraction and sphericity index. Transmitral flow pattern showed missing A-wave in dogs with atrial fibrillation and in all dogs the E-was was increased. In conclusion, dogs with DCM develop systolic and diastolic dysfunction quantifiable only through echocardiography. It is also recommended to evaluate the above mentioned measurements during disease progression.
Keywords: canine, myocardial dilatation, echocardiographic parameters

Canine idiopathic cardiomyopathy (DCM) is a primary cardiac disease affecting the heart muscle by fatty and fibrotic infiltration of the myocardium (11).

Symptoms can be dormants for years and morbidity happens when the dilatation and impaired contraction of the ventricles are responsible for the appearance of clinical signs.

Although echocardiographic and electrocardiographic (ECG) changes are already visible during the occult phase, this pathology is never diagnosed at this stage unless the dog is referred for a screening. Doberman Pinschers are benefiting from this examination due to the high prevalence of the disease in this breed. Other breeds like Cocker Spaniels, Great Danes, Irish Wolfhound and Newfoundlanders have been well studied because of their predisposition for DCM, however any giant or large to medium dog is susceptible to this cardiac condition.

After a few years DCM rapidly progresses to the third stage called overt phase where it is more prone to be detected due to the presence of congestive heart failure (CHF) accompanied by clinical signs such as exercise intolerance, dyspnea, effusions, cough and syncope (8). Even though the dog is already in the symptomatic phase, an echocardiogram is still required in order to establish the diagnosis of a

DCM, start the appropriate treatment, monitor the course of the disease and provide the owner with an adequate prognosis.

The purpose of this study is to collect and review echocardiographic and electrocardiographic data in dogs diagnosed in the overt phase of DCM, using as a reference the guideline released in 2003 by European Society of Veterinary Cardiology (ESVC) Taskforce (8).

Materials and methods

Case records of client-owned dogs referred to the Cardiology unit of the Veterinary Teaching Hospital of the Faculty of Iasi between January 2016 and November 2019 were selected from the archives.

Dogs met the inclusion criteria if they presented at least one sign of CHF during clinical examination in conjunction with a dilated left ventricle and decreased shortening fraction (FS < 30%) based on echocardiographic measurements.

Dogs were excluded if they were diagnosed with any significant systemic disease that could affect the cardiovascular system such as renal and endocrine disorders, congenital or acquired heart disease other than DCM or if they were receiving any cardiovascular drug before enrollment except for diuretics.

Baseline clinical history was gathered with a special attention to the cardiovascular and pulmonary systems. Information about breed, age, sex and body weight were recorded.

Diagnostic tests consisted of thoracic radiography, complete echocardiographic scan and a five-minute six leads electrocardiography.

Doppler echocardiography was performed by a single operator using the General Electric Logiq V5 Expert machine. Standards views were taken on right and left lateral recumbency on unsedated dogs using phase arrays transducers of 4-8MHz.

Each dog received a complete echocardiographic examination with a vigorous recording of a selection of parameters that were meant to be investigated for their relevance in the diagnosis of overt DCM. The selected parameters were grouped into 3 categories: cardiac dimensions, evaluation of the systolic function of the left ventricle (LV) and evaluation the diastolic function (LV). Certain values were normalized to the body surface area (1).

Cardiac dimensions were evaluated by the ratio between the left atrium and the aorta (LA/Ao) measured on a right parasternal short-axis view at the heart base; the left ventricular internal diameters in systole and diastole were obtained on M-mode indexed (LVIDSi and LVIDDi) and normalized to body-weight and surface area (4); the sphericity index (SI) was calculated using the ratio between the end diastolic left ventricle internal diameter in long axis measured on B-mode to short axis measured on M-mode (6).

Regarding the systolic function of the left ventricle, the end systolic and diastolic volume normalized to body surface area (ESV_I and EDV_I), ejection

fraction (EF) and fractional shortening (FS) were measured using Simpson's method of disk (SMOD). E-point-to-septal-separation (EPSS) was obtained on M-mode and represents the opening of the mitral valve depending of the blood flow.

Evaluation of the diastolic function of the left ventricle was realized by placing a sample volume at the level of the mitral valve leaflets, recording the Transmitral flow velocities (TMF) using a pulsed wave spectral Doppler echocardiography and measuring the peak E-wave velocity and E/A ratio when available.

The ECG was performed using 6 leads Polyspectrum 8E/8V on lateral recumbency and the ECG tracing was analyzed for 5 minutes to assess the entire rhythm and screen for atrial fibrillation (AF).

Lateral and dorsoventral thoracic radiographs were taken with the Examion CR Smart to evaluate the presence of pulmonary edema, cardiomegaly or pleural effusions.

Statistical analyses were performed using the IBM SPSS software. The data is presented as mean value and standard deviation for each parameter.

Results and discussions

A total of 21 dogs met the inclusion and exclusion criteria for overt DCM. There were 12 different pure-breed dogs and 1 cross-breed. Breeds were represented by German Shepherd (n=3), Labrador Retriever (n=3), Doberman Pinscher (n=2), Bucovina Shepherd (n=2), and one of each breed: Dog de Bordeaux, Central Asian Shepherds, Caucasian Shepherd, Alsatian Shepherd, Mioritic Shepherd, Schnauzer, Cocker Spaniel, German Shorthaired Pointer and mixed-breed. Those dogs were categorized into giant breeds (Bucovina, Central Asian, Mioritic and Caucasian Shepherds, Dog de Bordeaux), medium-large breeds (German Shepherd, Alsatian Shepherd, Doberman, Labrador, German Shorthaired Pointer, Schnauzer) and medium breed (Cocker Spaniel).

The male to female sex-ratio was 18:3 and mean body-weight was 46 kg, ranging from a 18 kg Cocker to a 83 kg Central Asian Shepherd. The mean age at time of diagnosis was 8 years old and ranged from a 3 years old Mioritic Shepherd to a 12 years old Labrador and German Shepherd.

Echocardiographic studies on the cardiac dimensions revealed that the mean LA/Ao was 2.1±0.5 (mean ± standard deviation) and 19 dogs (90%) had atrial dilatation, which is defined by a La/Ao ratio greater than 1.6 (3). Concerning the ventricular internal diameters, mean LVIDDi and LVIDSi were 1.9±0.3 and 1.4 ±0.7 respectively. Those values were above the reference range and are an indicator for ventricular enlargement. The rounded shape of the left ventricle secondary to its dilatation is expressed by a decrease in the sphericity index (SI). The mean SI obtained in the group was 1.2 which is lower than the minimal SI of 1.65 (Fig. 1).

The results of the evaluation of the systolic function demonstrated that all dogs had abnormal M-mode measurements. The assessment of volumes showed

values above 95 mL/m2 and 55 mL/m2 for EDVi and ESVi respectively and below 30% and 50% for the SF and EF, which goes together with the EPSS significantly higher than 6.5 (Table 1).

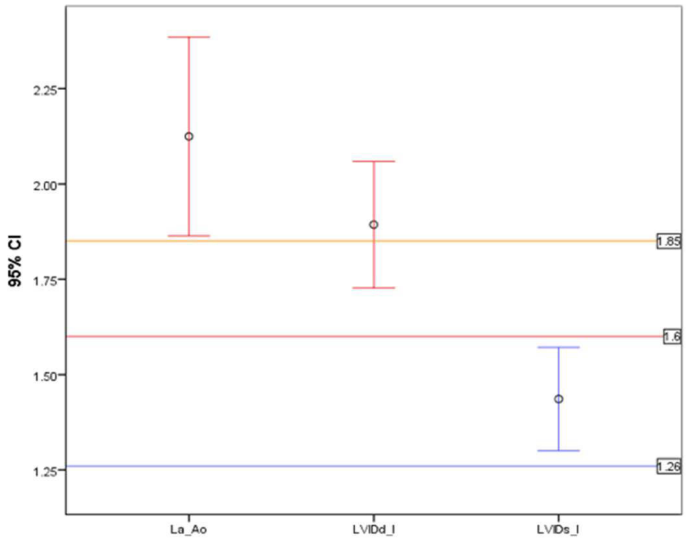

Fig. 1. Plot representing the 95% C.I. of selected measurements for the left atrium and ventricle in dogs with dilated cardiomyopathy. Enlargement of the cardiac chambers in dogs with DCM is measured by the increase size of the left ventricle in systole and diastole as well as the increase ratio between the left atrium and the aorta

Table 1

Shows a significant increase in the end diastolic and systolic volumes concomitant with the decrease of the fractional shortening and ejection fraction as well as an increase in the E-point-to-septal-separation

Parameter	Mean	SD
ESV_I	82.4	39.3
EDV_I	117.7	50.4
EF	33.0	13.8
SF	17.2	7.4
EPSS	12.1	1.9

Regarding the diastolic echocardiographic indices on Doppler-derived analyses, all of the dogs had an increased E wave velocity and E:A ratio on the transmitral flow pattern (TMF) with a mean value of 1.2±0.7 m/s and 1.8±0.7 respectively (Fig. 2).

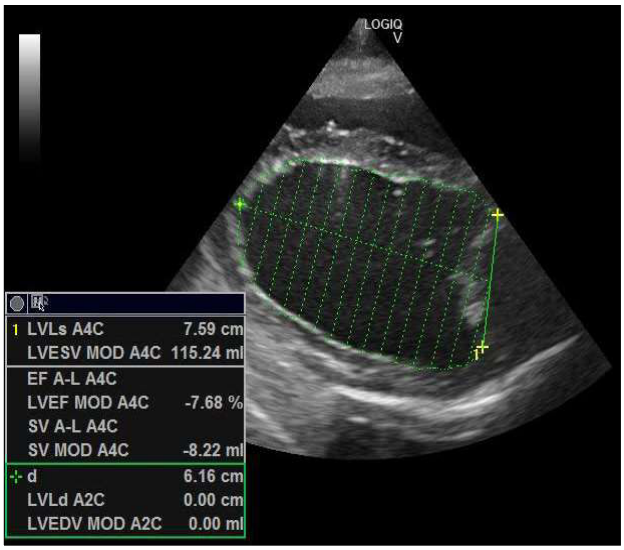

Fig. 2. Evaluation of the left ventricular diameter in diastole using SMOD

Atrial fibrillation was recorded on the ECG tracing of 60% of the dogs and ascites was the most common clinical presentation as it was found in 67% of the patients and was automatically accompanied by pulmonary edema and syncope. Syncope was reported in 3 dogs (Dog de Bordeaux, Cocker and mixed-breed) and pulmonary edema was evident on thoracic radiograph in 9 dogs.

In the early publications, Sandusky et al. (12) introduced DCM as an idiopathic cardiomyopathy and the diagnostic of dilated cardiomyopathy was confirmed by necropsy. Since that time, this disease has benefit from many researches and the ESVC Taskforce for Canine Dilated Cardiomyopathy has released a guideline in 2003 where they stated that the diagnosis of DCM was based on the identification of myocardial dysfunction and the most sensitive method for that was the echocardiography (7). Nowadays, the literature is rich with studies that investigated the different aspects of this pathology such as breed epidemiology, prognostic value of various clinical signs, survival time, breed specific echocardiographic parameters and more (2, 4, 6, 8).

With the increase availability of ultrasonography in the practices, echocardiography has become a standard imaging technique and therefore the use

of echocardiography should be valued as the best tool to diagnose DCM from other diseases with similar clinical signs. Our study collected and reviewed the most useful echocardiographic parameters in order to praise the importance of ultrasonography in the diagnostic of DCM. As a result for our retrospective investigation on 21 dogs in the overt phase, every parameter specific to the DCM was outside of the published normal range values (1, 4, 13).

Measurements of the cardiac dimensions by the mean of M-mode echocardiography gives the clinician a rapid and explicit idea about the presence and stage of the disease. The dilatation and hypokinesia of the ventricle is immediately visible on this examination. Those measurements offer valuable information about the prognosis since Martin et al found a negative association between increased internal diameters of the left ventricle (LVID) and survival time (9). They explained this finding by the fact that LIVD were related to the contractility and an increase in the diameter would affect the contraction of the cardiac chambers. In our study, the mean values for LVD-indexed in systole and diastole were above the normal values. The measurements of the internal diameters give information about volume overload, which is correlated to the value of the sphericity index (SI). This parameter reflects geometrical changes in the left ventricle as the heart becomes rounder when the internal volume increases. Although the authors Holler et al judged the SI to be inferior to the Simpson's Method of Disk (SMOD) when screening early echocardiographic changes in Doberman Pinschers (8), we found that the SI had an excellent specificity (100%) in our group of dogs presenting clinical signs of DCM. The SI has also been proposed by the ESVC Taskforce as a major criteria for the diagnosis of canine DCM (7). Moreover, we value this parameter as the LV diameter is evaluated using the right parasternal long axis-view on bi-dimensional echocardiography, which is technically less demanding and offers a clear view of the 4 chambers. This incidence is the easiest to visualize and it can be used by the practitioners as a tool of communication to show the owner the gross aspect of the cardiac silhouette, argument on the importance of treating DCM or justify the evolution of the disease such as the bad prognosis.

The thinning of the ventricular wall is associated with a decrease in the myocardial systolic function and the echocardiographic parameters that are used to evaluate this pathologic process present some major interests. Klüser et al have studied the predictors of sudden cardiac death (SCD) in Doberman Pinschers with DCM and they discovered that an alteration of the end diastolic volume indexed (EDV_I) by 50 mL/m^2 increased the risk of SDC by 8 times (8). During our study, we recorded a maximum value of 223.5 mL/m^2 in a Mioritic Shepherd and used that finding to warn the owners about the risk of SCD of their dog. According to the research of Borgarelli et al, the ejection fraction (EF) significantly affects the survival time and represents a more accurate index of global myocardial function compared to the shortening fraction (FS) (2) that is influenced by the presence of mitral valve insufficiency (5). However, either of those two parameters can be used as a major criteria for the diagnosis of DCM proposed by the ESVC Taskforce (4). Although the

ESVC Taskforce reviewed the EPSS as a minor criteria and the study about prognosis indicators found no relationship between an increase EPSS and longevity (2), Holler et al revealed that EPSS > 6.5 mm would be the value with the highest sensitivity (100%) and specificity (99%) to detect DCM. They also obtained a significant higher EPSS of 12.1±3.21 mm in dogs belonging to the DCM group (6), which is similar to our results with a mean EPSS of 12.1±1.9 mm.

In order to assess the ventricular diastolic function, we used the E-wave velocity and E: A ratio obtained by recording the transmitral flow (TMF) on Doppler echocardiography. This measurement reflects the pressure gradient between the atrium and the left ventricle as well as the compliance of the myocardium and its ability to relax. The results obtained in our group were increased compared to the reference values of 1.46 and 0.87 m/s assessed by Chetboul (3). This aspect of the TMF corresponds to a restrictive pattern, which is specific to dilated cardiomyopathy and is explained by the loss of compliance of the left ventricle together with volume overload. In their study about DCM, Borgarelli et al. (2) noticed that dogs with a restrictive TMF pattern presented more often severe clinical signs of heart failure (2). The abnormal diastolic function plays a major role producing the signs of the disease since the increased end diastolic pressure of the left ventricle leads to an increase in the pulmonary vein pressure and finally produces pulmonary edema.

According to previous reports, the incidence of atrial fibrillation (AF) in dogs with DCM ranges from 30% to 71% and tends to be more common in large to giants breeds, with half of them already presenting clinical signs of congestive heart failure (10). In our group AF was recorded in 60% of the dogs.

The extensive representation of giant shepherd dogs in our study reflects the popularity of those breeds in Romania. This is justified by their working qualities such as protecting livestock or guarding the house, but it also confirms the high prevalence of dilated cardiomyopathies among this breed category.

Along this study, we tested the scoring system proposed by the ESVC Taskforce for diagnosing DCM (7). By collecting the mean values of our echocardiographic and ECG findings, we attributed points depending on the importance of the criteria. Regarding the major criteria, 3 points were scored if the internal diameter, sphericity index and either the shortening or ejection fractions of the left ventricle were above the reference values established by the ESVC Taskforce. As every mean value were greater than the maximal value, each dog from our panel obtained a score of 9 points. To those were added 1 point per minor criteria such as increased EPSS, left atrial enlargement and atrial fibrillation, which made a total of 11 points for all the dogs and 12 points for 60% of the patients that presented AF. Those results were higher than the minimal score of 6 that should be obtain in order to establish a diagnosis of a DCM. Initially, this scale was designed to screen DCM but we noticed that the high score correlated well with the severity of the symptoms in our group, so this grading system could be reconsidered as an efficient tool of communication between practitioners or practitioners and owners. It

could help identifying a DCM and evaluate its progression, as the score should increase with the worsening of the condition.

Conclusions

By being the major cardiac disease in large and giant dogs, DCM needs an accurate diagnosis. Identification of the clinical signs such as dyspnea is the first step in the medical approach but the diagnosis should always be established by echocardiography. An echocardiogram is the best non-invasive method to assess the systolic and diastolic function, which is crucial in the follow up and treatment of this disease. Because of the outdoor lifestyle of large and giant breeds, the respiratory signs could be misinterpreted for a respiratory pathology, which makes the diagnosis of a DCM challenging. Our results encourage the general practitioners to use echocardiography in daily routine practice in order to diagnose the DCM earlier in its course and provide the dog with the best prognosis and life quality.

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STUDY ON DIAGNOSIS OF CHRONIC KIDNEY DISEASE IN DOGS AND CATS IN SOME VETERINARY CLINICS IN ROMANIA

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Summary

Chronic kidney disease (CKD) is defined as a functional and structural disturbance of one or both kidneys, that occurs during an extended period, usually three or more months. CKD is asymptomatic for a long time, even years, and when it becomes clinically apparent the structural and functional damages are irreversible and frequently progresses to fatal kidney failure. Dogs and cats of any age or breed can be affected, but most of the time the older animals are more likely to develop CKD than younger ones. There is no cure for CKD, but there are various treatments which can prolong pet's life for months to years, depending on the stage, so the faster the pet is diagnosed, the easier the treatment will be. There are various methods to diagnose CKD in small animals, including blood tests, urine tests, imaging tests and others, depending on the accuracy of the test and the financial possibility of the owner. The purpose of this paper is a screening on diagnosis of chronic kidney disease in dogs and cats in some veterinary clinics in Romania.

Keywords: chronic kidney disease, diagnosis, dog, cat

Chronic kidney disease (CKD) is defined as a functional and structural disturbance of one or both kidneys, that occurs during an extended period, usually three or more months.

Each kidney contains thousands of nephrons (which is the morpho-functional unit of kidney) that act like a complex filter for plasma. When more than half of the nephrons are damaged, the pet is no longer able to filter plasma and preserve water. Consecutively, large amounts of diluted urine will be lost and accumulation of toxins (eg. creatinine, urea, calcium, phosphates, electrolytes) will occur.

The kidneys help to regulate blood pressure, maintain homeostasis, and also produce erythropoietin which is necessary in synthesis of new red blood cells (14).

Etiology of CKD is usually difficult to determine because it is hard to identify which part of the nephron is affected (tubular, glomerular, interstitial, vascular or mixed) (6). From the epidemiological point of view, the prevalence of this disease increases 1-3% after 12 months of life. Based on the study of Jepson et al., 2009, CKD especially affects geriatric animals: 10% of dogs and 35% of cats (2).

Chronic kidney disease is classified in four stages and it usually progresses lethally. Staging is based initially on fasting blood creatinine or fasting blood SDMA

(specific marker of CKD) concentration or both assessed on at least two occasions in a hydrated, stable patient. The dog or cat is then substaged based on proteinuria and blood pressure. The pet may show: polydipsia, polyuria, hypertension, lack of appetite, gastrointestinal disturbances, anemia and general weakness (2).

Stage 1 is the nonazotemic stage, which is often discovered by accident during a routine examination and consists in decreasing capacity of concentrating the urine and possible proteinuria. In stage 2, besides polyuria and proteinuria, polydipsia may occur, and the clinical signs are minimal. Stage 3 is characterized by a moderate azotemia and systemic clinical signs may be present. Stage 4 resembles to the previous stage, but the manifestation is more severe, with dehydration, anemia, and malnutrition (12). A common sign for all the stages is the increased systolic blood pressure from prehypertensive to severely hypertensive, proportionally damaging the target organ (2, 7).

CKD is not a curable disease, but there are treatments designed to diminish the work the kidneys need to perform, to reduce wastes generated by protein metabolization and to increase essential substances that may be low (such as potassium) (14).

The diagnosis is based on various tests which can indicate an increased blood waste production and abnormalities in the urine, such as proteins or a high level of electrolytes. Early diagnosis of CKD corelated to appropriate treatment have the greatest potential of stabilizing or improving renal function and the possibility to extend pet's life (8).

Materials and methods

The study was performed on eight animals: three cats and five dogs, of 4 to 17 years old, registered in three clinics from distinct areas in the north-western part of Romania (Timișoara, Satu Mare). Two members of the group were females and the other six were males. The breed spectrum was large, including: three half-breed dogs, two European cats, one Cocker spaniel, one German Sheppard and one Siamese cat. The basic data for patients are presented in Table 1.

The animals were presented to the veterinarian for various reasons, mainly general state alteration and disturbances of urination. Anamnesis and clinical examination leaded to chronic kidney insufficiency diagnosis. In order to have an accurate diagnosis some paraclinical tests were performed.

Table 1

Basic data for investigated animals

Patient name	Species	Age	Sex	Breed
Rex	dog	15 y	M	German Sheppard
Goigli	dog	12 y	M	Cocker Spaniel
Jessy	dog	7 y	F	Half-breed
Aldo	dog	11 y	M	Half-breed
Zdreanta	dog	14 y	F	Half-breed
Paxi	cat	13 y	M	European
Max	cat	4 y	M	European
Francois	cat	17 y	M	Siamese

The paraclinical methods generally used for diagnosis of CKD are the following:

1. Urinalysis

Urine strips is the most common diagnostic tool used to determine imbalances of pet's organism. The dipsticks contain ten different reagents which react with the urine sample, by changing color. This analysis is used to underline the presence of: urobilinogen, glucose, bilirubin, ketones, blood, protein, nitrite, leukocytes and also measure specific gravity and pH. The test method consists of immersing the test strip in a well-mixed urine sample for a short period of time, then extracting it from the container. The strip is then left to stand for 1-2 minutes necessary for the reaction to occur. Result interpretation can be done by comparing the pad color with a chromatic scale provided by the manufacturer (5). The presence of protein or blood associated with high specific gravity of the urine indicate the occurrence of CKD.

Urine strips method is the cheapest and most accessible of all the methods and it takes very short time to proceed and interpret. The method is less specific and not very precise (an improper technique can produce false results) so a secondary test is required (1).

Urine microscopy is used to detect the presence of cells, crystals, microorganisms and tumor cells. The urine sample is centrifuged and the sediment is analyzed under the microscope. The urine sediment can be catalogued in two classes: organized (minerals and organic elements) and unorganized (epithelial cells, leukocytes). This method can be used to certainly confirm the alteration of the kidney caused by the excess of organic elements (2).

Urine microscopy is an easy, fast and cheap method, helpful in monitoring the medical condition. The result can be interfered by certain medicines which can alter the appearance of urine and there could also be errors of misinterpretation. For females, menstrual blood and vaginal medicine can disrupt the result (9).

2. Blood tests

Biochemical analysis is a basic metabolic panel which measures many parameters including creatinine, blood urea nitrogen and minerals, with high importance in diagnosis of CKD (2).

Serum creatinine derives from creatine phosphate (byproduct of muscle metabolism) and is an important marker of glomerular filtration rate (GFR). There is an exponential relationship between creatinine and GFR so that in early CKD there can be large changes in GFR with relatively small changes in creatinine concentration (5). It's important to know that many breeds of dogs and cats have higher serum creatinine concentration because of their increased muscle mass (Siamese cats, Cocker spaniel dogs) (4). Serum creatinine has variable values, depending on the species and stage of the disease, represented in table 2 (7, 10).

Table 2

Chronic kidney disease staging and monitoring creatinine level

Azotemia (creatinine level mg/dl)	Species	**Normal**	**Stage 1** Markers of CKD present	**Stage 2** Mild azotemia	**Stage 3** Moderate azotemia	**Stage 4** Severe azotemia
	Dog	0.3-1.3	<1.4	1.4-2	2,1-5	>5
	Cat	0.8-1.8	<1.6	1.6-2.8	2,8-5	>5

Creatinine is produced endogenously and excreted by urine. Its clearance (volume of plasma that will have to be filtrated by the glomeruli per minute) can be used to estimate the GFR in steady state (2).

Blood urea nitrogen (BUN) is produced in the urea cycle as a waste product of protein digestion. The production and excretion of urea does not proceed a constant rate, so the urea clearance is not a reliable estimate of the GFR (decreased urea clearance may occur without a GFR decrease). Normal BUN concentrations are 8-25 mg/dl in canines and 13-35 mg/dl in felines (2).

Phosphate and ionized calcium are also markers in early kidney disease and changes in concentration will not be seen until later stages. The extent of which electrolytes appear in the urine is the net result of tubular reabsorption and secretion. Pets suffering of CKD usually have increased blood phosphorus. As the kidneys fail the amount of parathyroid hormone (PTH) in the body is elevated and the amount of vitamin D is reduced. Elevated PTH itself may be responsible for some of the signs shown by pets with CKD: weaken bones that can lead to fractures (14).

Biochemical analysis gives accuracy and reliability in diagnosis of CKD. The biochemical analysis costs are higher than the procedures presented below and some owners don't have disposable financial resources or are not willing to pay the cost. Also, it takes more time than a simple urinalysis.

Symmetric dimethylarginine (SDMA) results from the intranuclear methylation of L-arginine and is released in cytoplasm after proteolysis. SDMA is

excreted by the kidney and reflects GFR more accurately than creatinine (3, 11). SDMA increases as early as 25% loss of kidney function, making SDMA more reliable in both acute or active kidney injury and chronic kidney disease. Creatinine cannot identify kidney issues until almost 75% of kidney function is lost (8). Results of a retrospective longitudinal study that includes 21 cats and 19 dogs with CKD show that SDMA increased on average 17 months (for cats) and 9.8 months (for dogs) earlier than serum creatinine (8).

SDMA is an earlier indicator of progressive kidney function loss, often increasing before other parameters and provides useful information for treatment management. It is a more reliable and sensitive indicator of kidney function than other tests and detects as little as 25% loss of function. Unfortunately, the SDMA test for dogs and cats by Idexx Laboratories became available in Europe only in 2016 (13). As a consequence, in Romania it is not a usual method of diagnosis because of its expensive price. Therefore, there is a medical analysis laboratory in Bucharest where the veterinarians can send a blood sample for examination (15).

Hematology (CBC- complete blood count) can evaluate numerous conditions involving blood and its components: red blood cells (RBC), white blood cells (WBC) and platelets (THR). It's used to highlight the presence of anemia, verify blood condition, see how medication is affecting blood cells and detect other health issues. A sample of blood is collected by inserting a needle into a vein and then analyzed by a hematology apparatus (3). The normal blood count values are presented in Table 3 (16).

Table 3

Normal blood count values for adult dogs and cats

Parameter	Canine	Feline
WBC (/µL)	6-17	5-19
RBC count (X10 ^{6}/µL)	5.5-8.5	5-10
Hb (g/dL)	12-18	6-15
Hct (%)	37-55	24-45
MCV (µm^3)	60-77	40-55
MCH (pg)	20-25	13-18
MCHC (%)	32-36	31-36
Neu (%)	50-80	40-80
Ba (%)	0,5	0.5
Mon (%)	2-10	2-6
Eo (%)	1.6-7.5	2-12
Lym (%)	10-30	5-30
THR (X10 ^{6}/µL)	200-900	300-700

MCV=mean corpuscular volume, MCH=mean corpuscular hemoglobin, MCHC= mean corpuscular hemoglobin concetration, Neu= neutrophils, Ba= basofiles, Mon= monocites, Eo= eosinophils, Lym= Lymphocytes.

The parameters involved in diagnosis of CKD are: red blood cells (RBC), hemoglobin (Hb), hematocrit (Hct) and thrombocytes (THR). RBC below range can indicate anemia, which is common in CKD. Its main cause is inadequate production of erythropoietin by the diseased kidney. Hb and Hct below the range are also correlated to anemia. Low THR is associated with low RBC in CKD (2).

Hematology test is reliable and reproductible and it can be used as an additional information in diagnosis of CKD. It is quite expensive for the little information related to this disease, consequently this test alone cannot indicate 100% the presence of CKD and can lead the veterinarian to a wrong conclusion (17).

3. Imaging tests

Ultrasonography is a diagnostic imaging technique based on the application of ultrasound and is used to show an image of internal organs and tissues. This diagnosis method's aim is to find the source of disease, to evaluate kidney structure, topography and dimension changes. The ultrasound pulses echo off tissues with different reflection properties and are recorded and displayed as an image. In correlation with CKD, ultrasound provides information about kidney structure abnormalities, evidenced by increased echogenicity (2). Sometimes, the presence of renal or bladder lithiasis can be remarked. Irregular shaped kidneys, cortical echogenicity, papillary calcifications, poor visibility of the renal pyramids and the renal sinus suggest CKD (5).

The device is portable, simple to use and it provides images in real time. Ultrasonography does not use harmful ionizing radiation, and has lower costs than other imaging modalities. This method singular can not certainly diagnose CKD, but it can add supplemental information to prognosis. Besides, it requires a skilled operator to avoid misinterpretation.

Radiography is an imaging technique using gamma rays and X-rays (ionizing radiation) to view and produce pictures of body's internal structures. A certain amount of the radiation is absorbed by the tissue or organ, dependent on its density and composition. Radiologic examination of urinary apparatus consists of two phases. The first stage is based on a ventro-dorsal radioscopy after digestive system's content is evacuated. The second stage requires administration (intravenous or by vesical catheterization) of a contrast material to help improve the visibility of specific organs and then execution of Rx. Excretion of contrast material ensures a verification of kidney's function (propagation, permeability, filling and evacuation) and it offers quick imaging (9).

The inconveniences of this method are that it uses ionizing radiation which can be harmful (because it mutates cells which causes cancer) and there is a limited number of X-rays that can be done in a period of time. Also, adverse reactions to the contrast material have been reported in dogs and cats (vomiting and hypotension) (1). There are no specific imaging features for CKD and the price is higher than ultrasonography.

4. Blood pressure appreciation

Blood pressure is a measure of the force that heart uses to pump blood around body. It is measured with a sphygmomanometer and is expressed in mmHg. In small animals, the cuffs are applied on femoral, tibial or coccygeal artery in order to compress it and measure blood pressure. The normal blood pressure ranges between a maximum limit (systolic pressure) and a minimum limit (diastolic pressure). Systemic hypertension is present in 20 to 30% dogs and cats with CKD. Factors contributing to hypertension include renal ischemia associated with CKD, that result in the renin-angiotensin system and increased sympathetic nervous system activity (3). Clinical and pathological manifestations of systemic hypertension include ocular abnormalities (retinal detachment and hemorrhages) and cardiovascular abnormalities. Once the diagnosis of CKD is certain, the first step in improving renal function is to reduce hypertension with angiotensin-converting enzyme (ACE) inhibitors, and slow the progression of CKD. Angiotensin is a hormone which contributes to intraglomerular hypertension and proteinuria, and that's the reason why ACE inhibitors are administrated: to decrease the filtration of protein by lowering intraglomerular hydrostatic pression (2, 3).

Hypertension is an important indicator of CKD; it allows taking multiple readings over an extended period of time because it is a simple and quick method. Blood pressure appreciation is a quite difficult technique to apply on dogs and cats because of the reaction the animal may have during the vascular compression procedure. Errors might occur because of "white-coat" hypertension (animals tend to stress out when they enter a veterinary cabinet). Effort can also give false hypertension, so measurement has to be repeated after ten minutes (3).

Results and discussions

The anamnesis for the patients presented in clinics revealed clinical signs like weakness, lack of appetite, weight loss, polyuria and gastro-intestinal disturbances, but there were cases without clinical signs of renal perturbance.

Considering the cases history (diet, habitat, other diseases) and clinical evidence, additional investigations were done. The chosen tests were dependent on the endowment of the clinic and the willingness of the owner and are presented in table 4. Unfortunately, for some of the animals CKD was discovered in a very late stage and some of them passed away.

According to Table 4, biochemical analysis and ultrasonography were performed for all the patients to diagnose CKD. The biochemical analysis provided valuable information about serum parameters (BUN, CREA), with high importance for kidney's disease (Table 5). Ultrasonography illustrated morpho-physiological disturbances and provided images in real time.

Table 4

Tests used to diagnose CKD

Test	Paxi	Aldo	Zdreanta	Max	Goigli	Jessy	Francois	Rex
Urine strips	-	✓	✓	✓	-	-	✓	-
Urine microscopy	-	✓	✓	-	-	-	-	-
Biochemical analysis	✓	✓	✓	✓	✓	✓	✓	✓
Hematology analysis	-	✓	✓	-	-	-	-	-
SDMA	-	-	-	-	-	-	-	-
Ultrasonography	✓	✓	✓	✓	✓	✓	✓	✓
Radiography	-	-	-	-	-	-	-	-
Blood pressure	✓	-	-	✓	-	-	-	-

Also, urine strips had a high rate of usage (four cases), but there were done in only one of the clinics involved in this study, so the application of this test depended on the doctor's way of approaching the case and on the owner's preferences since it is the cheapest and most accessible test.

In four cases out of nine were used either urine microscopy, hematology analysis or blood pressure measurement. These tests don't have the same importance in diagnosis as the other ways of measurement, but they bring some supplementary information about the presence of anemia, hypertension and urinary crystals, which can complete the clinical picture of CKD.

Table 5

Details and values of CREA and BUN in patients with CKD

Patient name	Species	Age	CREA	BUN
Rex	dog	15 y	1	43.1 ↑
Goigli	dog	12 y	3,8 ↑	43,5 ↑
Jessy	dog	7 y	8,7 ↑	>200 ↑
Aldo	dog	11 y	13.3 ↑	>130 ↑
Zdreanta	dog	14 y	>13.6 ↑	>130 ↑
Paxi	cat	13 y	3.3 ↑	58.2 ↑
Max	cat	4 y	7.7 ↑	76 ↑
Francois	cat	17 y	2.8 ↑	63.7 ↑

The applied tests revealed that some of the patients were suffering not only of CKD, but also of FIV (feline immunodeficiency virus), Babesiosis, pyometra and gastritis.

Radiography and SDMA were used in neither of the cases because they require special equipment and training. Radiography can be applied in certain

conditions as a 24 hour fasting before the test and it also needs contrast substances to obtain a significant result. SDMA equipment is very expensive so not many cabinets can afford it; but it provides the most conclusive results in diagnosis of CKD since it measures a specific parameter involved in both early and lately stages.

Conclusions

The diagnosis of chronic kiedney disease in the three clinics from this study was based on the anamnesis, clinical and paraclinical investigations. The type and number of the performed tests were dependent on the clinic's equipment and the owner's financial background and willingness to make additional investigations.

The common used tests for diagnosis were biochemical analysis and ultrasonography, which were enough and effective in diagnosis of chronic kidney disease. Urine strips were frequently used, but they were not conclusive enough for chronic kidney disease, although it can lead the doctor to suspicion of renal disturbance.

For additional information, tests like blood count, blood pressure measurement and urine microscopy have been used in some cases to confirm associated diseases.

Chronic kidney disease was discovered in a lately stage because the clinical signs are not evident in the early ones and the creatinine level cannot identify kidney issues until almost 75% of kidney function is lost. Because SDMA test can detect as little as 25% loss of function, our recommendation it to use SDMA as for a veterinary routine diagnosis test.

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THE EPIDEMIOLOGICAL INQUIRY IN CARNIVOROUS DERMATOPHYTOSIS AND THEIR ROLE IN ANIMAL AND HUMAN TRANSMISSION

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Summary

Carnivorous dermatophytoses affect the superficial skin layers, hair and claws. The most frequently incriminated pathogenic agent is *Microsporum canis.* It is possible the transmission between different animal species, even in humans. The study was conducted over a 2-year period and followed a survey of 8 episodes of dermatophytosis that involved 45 cats, 22 dogs, 2 goats and 27 owners from Timis County. The samples were performed by mycological exams. There were investigated the etiological agent as well as the animals` origin, living condition, age, other animals infestation and cross-transmission. We corelated the information from epidemiological inquiry, clinical examination and laboratory investigations. The results of the study revealed an increased prevalence in stray cats, young individuals and animals kept outdoor constanly. The *Microsporum spp.* was the only dermatophyte involved in skin lesions and asymptomatic skin. Infected stray cats or purchased pet shop dogs, the asymptomatic infected animals who cohabit with healthy ones represent the main rezervoir of dermatophytosis infestation.

Keywords: dermatophytosis, epidemiology, carnivorous, zoonotic risk

Dermatophytes infection is a type of fungal infection that affects the superficial skin layers, hair and claws. The species incriminated in the production of the cutaneous lesions are dermatophytes (*Microsporum, Trichophyton, Epidermophyton*) as well as the nondermatophytes (*Candida* and *Malassezia*) (13).

Dermatophytosis represents a serious and common contagious skin disease in dogs and cats. The significance of this disease for pet owners is based on the zoonotic potential. The prevalence varies with climate and local dermatophyte infestation (1).

Carnivorous dermatophytoses are sporadic diseases. The most frequently incriminated pathogenic agent is *Microsporum canis*. The transmission of the disease is dependent on many factors, inclusively the amount of infectious material, frequency of exposure, immunity and physiological stress of the animal.

There is a possibility of transmission between different animal species, even in humans. The contamination sources are represented by the infected animals, those with clinical signs and even those with subclinical signs, by the asymptomatic carriers, by different grooming objects or by the environment in which the infected animal lives and the means of transport.

Dermatophytosis is a very contagious disease because, both on the hairs and in the environment spores can survive for months, even years.

The high prevalence of *Microsporum spp*. in animals, especially in cats, has been reported in many studies. The veterinary doctor and the human one show a particular interest in the zoonotic risk of dermatophytosis (4, 7, 14).

In this context, the aim of this paper was to identify the dermatophytosis in carnivorous skin and their epidemiological implications in other animals, as well as in owners.

Materials and methods

The study was conducted over a 2-year period and followed a survey of 8 episodes of dermatophytosis. We examined 45 cats, 22 dogs, 2 goats and 27 owners from Timis County.

The steps of medical procedure were anamnesis, clinical examination and laboratory diagnosis. The hair samples were inoculated in Dermatophites Test Medium plate (DTM) and incubated at 25°C for 3-14 days. Growing fungi were identified by morphological characteristics.

We investigated the etiological agent as well as the animals` origin, living condition, age, other animal's infestation and cross-transmission. We asked the owner about the source of infestation, ways of infestation, living condition, and the presence of skin lesions in other animals or to themselves. We correlated the information from epidemiological inquiry, clinical examination and laboratory investigations.

Results and discussions

The epidemiological inquiry revealed the results as follows (Fig.1, 2, 3, 4).

Group 1 – 45 cats

Living conditions

1. Household cats reared indoors (kept indoor constantly) – 4/45 (9%),
2. Stray cats – 16/45 (35.5%),
3. Household cats reared outdoors (kept outdoor constantly) – 25/45 (55.5%).

Age

1. 2 months-1 year old – 24 cats/45 (53%),
2. 1-2 years old – 17 cats/45 (38%),
3. 2-3 years old – 4 cats/45 (9%).

Cohabitation with other animals

1. With cats – yes,
2. With dogs – yes,
3. With goats – yes,

4. With owners – yes.

Clinical signs

1. With skin lesion – 34/45 (76%),
2. Without clinical signs – 11/45 (24%).

Living conditions for symptomatic cats (34)

1. Stray cats – 16/34 (47%),
2. Household cats reared outdoors – 16/34 (47%),
3. Household cats reared indoors – 2/34 (6%).

Living conditions for asymptomatic cats (11)

1. Household cats reared outdoors – 9/11 (82%),
2. Household cats reared indoors – 2/11 (18%).

Group 2 – 22 dogs

Living conditions

1. Kept outdoors – 20/22 (91%),
2. Purchased from pet shop and kept indoor constantly – 2/22 (9%).

Age

1. 3 months-1 year old – 13/22 (59%),
2. 1-3 years old – 7/22 (32%),
3. 3-5 years old –2/22 (9%).

Cohabitation with other animals

1. With cats – yes,
2. With dogs – yes,
3. With goats – yes,
4. With owners – yes.

Clinical signs

1. With skin lesion – 12/22 (55%),
2. Without clinical signs – 10/22 (45%).

Living conditions for symptomatic dogs

1. Kept outdoors – 10/22 (45%),
2. Purchased from pet shop and kept indoor constantly – 2/22 (9%).

Living conditions for asymptomatic dogs

1. Kept outdoors – 10/22 (45%),
2. Purchased from pet shop and kept indoor constantly – 0.

More epidemiological data

- One out of two goats that live with cats and dogs presented skin lesions.
- 13 out of 27 owners had skin injuries (48%).

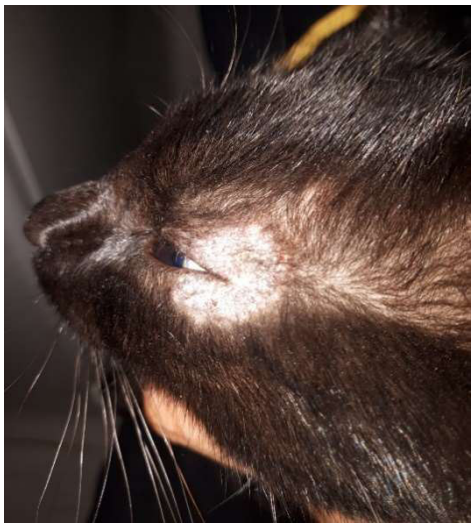

Fig. 1. *Microsporum spp.* – cat

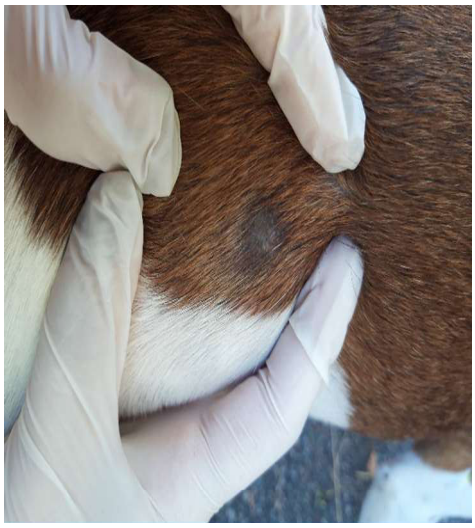

Fig. 2. *Microsporum spp.* – dog

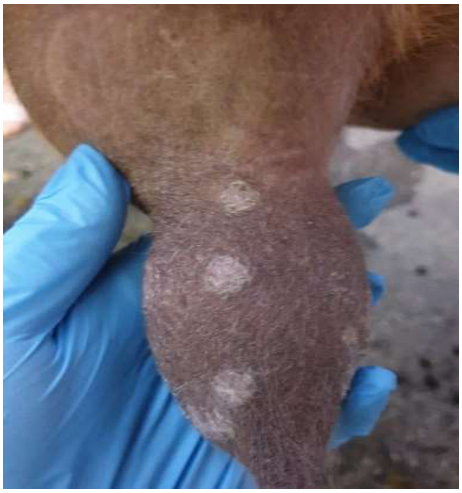

Fig. 3. *Microsporum spp.* – goat

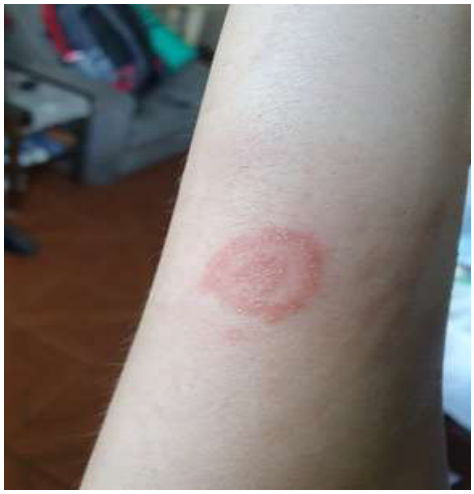

Fig. 4. *Microsporum spp.* – owner

The **laboratory exams** confirmed only the presence of the macroconidia belonging to the genus *Microsporum* as follows:

1. Symptomatic cats – 34/34 – *Microsporum* present
   1.a. Stray cats – 16/16,
   1.b. Cats kept outdoors – 16/16,

1.c. Cats kept indoors – 2/2.

2. Asymptomatic cats - 8 /11 - *Microsporum* present
   2.a. Cats kept outdoors – 6/9,
   2.b. Cats kept indoors – 2/2.

3. Symptomatic dogs – 12/22 – *Microsporum* present
   3.a. Kept indoors – 2/2,
   3.b. Kept outdoors – 10/20.

4. Asymptomatic dogs - 0/10 - *Microsporum* present

5. Two goats – one symptomatic and one asymptomatic – *Microsporum* present

6. The symptomatic owners – 13/13 - *Microsporum* present

Dermatophytosis is a frequent cutaneous infection affecting the keratinized tissues of humans, pets, and livestock. Animals can carry dermatophyte elements asymptomatically and are considered to play an important role in the epidemiology of the disease. As exposure to any infected lesion free animals, especially cats, may lead to the development of infection in humans (12).

Dermatophytosis in carnivores remains an important pathological concern, with an increased prevalence in cats, young individuals and zoonotic risk in pregnant ones (10).

The measures to control dermatophytoses have become important as raising dogs and cats indoor has become popular, making the contact between pet animals and humans more common (11).

Herein, we discuss the results of our recent investigations into the prevalence of dermathophytosis in cats and dogs, the investigations concerning the living conditions, age, cohabitation with other animals and humans, presence/absence of the clinical signs.

Regarding the results obtained from epidemiological investigations in a group of 45 cats, we note the presence of clinical signs in a number of 34 of them (76%), 32 of them belong to 2 groups – cats kept outdoor and stray cats. All stray cats had lesions and they were infected with *Microsporum spp.* All symptomatic cats were infected with dermatophytes.

A study performed in Iran showed that 14.5% of the 103 examined asymptomatic cats were positive for dermatophytes elements on direct examination (12).

Concerning the isolation of *Microsporum spp.* from the asymptomatic cats skin, we revealed the presence of dermatophytes in 8 cats from 11 (73%).

A study performed in Brazil investigated the prevalence of dermatophytes in

dogs, cats and environment floor through molecular epidemiology tools to identify the genetic profile of these infectious agents. *M. canis* was the only dematophyte species isolated from symptomatic, asymptomatic and environmental animal samples (5).

In order to study the presence of keratinophilic fungi with special reference to dermatophytes on the coat of dogs and cats living in the cities of Mexico and Nezahualcoyotl in the Metropolitan area of Mexico City, two hundred samples were collected from dogs and one hundred from cats. The most common fungi isolated were *Trichophyton terrestre*, *Microsporum gypseum* and *M. canis.* Keratinophilic fungi were found in higher number in the cat haircoat than in the dog's. This may represent a health risk for humans in contact with a dermatophyte infected cat or dog (8).

A descriptive study identified the risk factors for feline *Microsporum canis* infection at shelter intake. The feline population at the study shelter had a high prevalence of *M. canis.* Applying the results of this study to shelter protocols could optimize diagnostic approaches and shorten the length of stay for shelter cats and kittens, resulting in streamlined shelter operations and improved feline welfare (6).

The cohabitation between infected and noninfected animals proved that the reservoir of *Microsporum spp*. could be the cats, especially the asymptomatic ones who have the *Microsporum spp.* spores in the coat. The other cats, the dogs, the goat and the owners were infected and some of them had the clinical signs.

Multiple exposures to stray cats caused infection of mainly young female soldiers performing guarding duty. Other persons were infected by person-to-person transmission. These findings aided in the termination of the outbreak (2).

In our study, 12 out of 22 dogs (55%) presented skin lesions. The *Microsporum* spores were identified on the skin of all symptomatic dogs - kept outdoor (10/12) and 2 purchased from pet shop and kept indoor constantly. In this context, the cohabitation with infected animals and purchasing dogs from pet shop definitely represent the main infestation way.

The studies performed in Japan revealed that the feline and canine dermatophytosis incidence continues to be high. There appears to be two main routes in cats, namely, pet shop and outdoor due to strays, whereas for dog, the main route is thought to be through pet shops. Regarding the outdoor for cats, *M. canis* reservoir may form locally in certain regions and then spread sporadically. Therefore, when cases of outdoor - related infections, such as infected stray cats or pet cats with dermatophytosis known to be among a stray cat community, are diagnosed by veterinarians, attention should be paid to the probable existence of reservoirs of infections and the risk of subsequent outbreaks (15).

Our results reveal that the reservoir of infestation for goat (with/without skin lesions) and owners (13/27) was the infected cats who they have cohabited with.

Human cases of dermatophytoses are occasionally transmitted from animals and suffered from tinea corporis and sometimes kerion celsi. Many cases are reported in Japan and the most frequent causative agent of these diseases is

Microsporum canis. Feline dermatophytosis should be prevented and promptly treated since it is easily transmitted to people from cat lesions (9).

In our study, the infestation prevalence is high in puppies up to 1 year of age (24/45, 53% cats and 13/22, 59% dogs).

A total of 424 animals (268 dogs and 156 cats) with skin lesions (alopecia and peripheral scaling) were examined in southern Italy. *Microsporum canis* was the most common dermatophyte isolated from dogs and cats (77.7%), followed by geophilic dermatophyte species (*M. gypseum*, *Trichophyton terrestre*). Young dogs and cats, especially the 1-year old ones, showed a statistically significant higher prevalence of *M. canis* infection compared to older animals (3).

Conclusions

Dermatophytosis produced by *Microsporum spp*. is one of the most important skin diseases in carnivorous based on zoonotic risk and an increased prevalence in stray cats, young individuals and animals kept outdoor constantly.

Infected stray cats or purchased pet shop dogs, the asymptomatic infected animals who cohabit with healthy ones represent the main reservoir of dermatophytosis infestation.

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PARACLINICAL RESEARCH IN THE FELINE LOWER URINARY TRACT DISEASE

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Summary

Feline lower urinary tract disease (FLUTD) is diagnosed quite frequently among the feline population and is characterized by haematuria, dysuria, frequent urination, stranguria, periuria and urethral obstruction, which mainly affect the sedentary apartment cats. This morbid entity has a high incidence of diseases of the urinary tract in cats, which is often complicated by bacterial, fungal or parasitic infections at this level. Also, phenomena of urolithiasis, trauma, neurological or iatrogenic disorders of the urinary tract are commonly encountered. Following clinical studies it has been found that in 50% to 65% of the animals with urinary problems a precise etiological identification is not possible because the causes are multifactorial and complex, classifying as idiopathic cystitis. In this sense, we performed clinical and paraclinical investigations (blood biochemistry and ultrasound) on a group consisting of 15 cats, divided into different age categories: 2-4 years, 5-8 years and 9-11 years. In order to diagnose the conditions of the feline lower urinary tract disease, it is advisable to use in addition to the clinical methods of semiologic type, the paraclinical methods: blood biochemistry and ultrasound.

Keywords: cats, urinary tract, creatinine, urea, ultrasound

Feline lower urinary tract disease (FLUTD) are common in the clinical routine of this species due to the various symptoms characterized by haematuria, dysuria, frequent urination, strangulation, periuria and urethral obstruction, which mainly affect sedentary cats, who live at home and have a low water consume (1).

This morbidly entity is one of the most common diagnoses in feline medicine, which results from bacterial, fungal or parasitic infections of the urinary tract, from anatomical abnormalities of the urinary tract, different types of urolithiasis, neoplasms or traumatic, neurogenic or iatrogenic causes (7).

However, 50 to 65% of the affected animals it is not possible to identify the precise etiology, being multifactorial and complex, being classified as idiopathic cystitis (5).

Hematuria, polakisuria and stranguria are the clinical signs characteristic of feline lower urinary tract disease, but the specific cause of this syndrome is not often identified, sometimes due to associated conditions: infections, neoplasias, trauma, urethral obstruction, urolithiasis and sterile cystitis (6) .

As for the formation of uroliths, it is believed to be induced by industrialized dry food, rich in calcium, magnesium and phosphates. To avoid crystalline precipitation and the formation of obstructed urinary stones, maintaining a urinary

acid pH is more important than controlling the intake of magnesium or phosphates, as these crystals have low solubility at pH> 6.45. However, most cases of urethral obstruction in cats are related to urethral plugs, which are usually composed of a combination of protein material (Tam Horsfall mucoproteins) and crystals, but occasionally are made of organic matrix, or aggregates mineral crystal (3).

Largely, lower urinary tract disease affect male, obese, sedentary, dry-fed cats, living indoors, where they live with other animals and have a single source of drinking water. It has been shown that compared to short-haired domestic cats, the risk is lower for Siamese and long-haired species, probably due to their racial character, such as lethargy and obesity in Persian cats. Another predisposing factor is stress, similar to human interstitial cystitis (HIC), feline interstitial cystitis has neuroimmunoendocrine basis. This fact also refers to recurrences in 35 to 50% of felines (1).

Affected cats have haematuria, polachisuria, dysuria or stranguria, bladder distension and signs of uremia, such as vomiting, anorexia, lethargy, weakness and even anuria (2).

Urine analysis shows intense hematuria, mainly due to distension and inflammation of the bladder, variations in urinary pH and the presence of inflammatory cells, bacteria and / or crystals. During obstruction, serum creatinine and uremia concentrations will increase. Also, metabolic acidosis may be present in some animals. Uroculture should be performed to exclude or identify urinary tract infections in cats with suspected struvite disease, although the infection, as early as the first episode, is unlikely to occur (4).

In the case of lower urinary tract disease, the prognosis depends on the time of obstruction, complications and severity. Azotemia and the consequences of renal failure are the major death factors among affected cats (1).

Materials and methods

The research was carried out in the CLHC laboratory of the Faculty of Veterinary Medicine in Timisoara, in the discipline of Clinical and Clinical Lectures on Species, in the Bioclinic laboratories and in a private veterinary medical office in Timisoara on a number of 15 cats.

The cats included in the study were from different breeds, they are both female and male, ranging in age from 2 to 11 years.

In order to perform the blood biochemical examination, blood was collected from the antebrachial cephalic vein in vacuoles with EDTA, respectively activating gel for biochemical parameters.

In order to perform the ultrasound examination of the urinary tract, the mechanical toilet and sanitation with sanitary alcohol were previously performed, then an ultrasound gel layer was applied, the cats being contained in the dorsal decubitus on the ultrasound table.

The ultrasound images were obtained with a stationary X Vision My LabTM70 Vet ultrasound with a linear probe with a frequency between 9-18 MHZ and a portable ultrasound, model Mindray, DP-2200 Vet.

Statistical interpretation was performed using the IBM SPSS22 software, calculating the mean and dispersion indices for the analyzed parameters and testing the significance of the differences between the mean values was performed using the ANOVA and Turkey software.

Results and discussions

In the case of urinary tract disorders, it is imperative to biochemically investigate urea and serum creatinine, corroborating these aspects with the serum level of total proteins.

The average and dispersion indices of creatinine, serum urea and serum proteins in cats aged 2 to 4 years are shown in Table 1.

Table 1

Average and dispersion indices of some blood biochemical parameters in cats between the ages of 2 and 4 years

Specification		Average	Medium error of the average	Standard deviation	The value	
					Minimum	maximum
Creatinine (mg/dL)	M+F	**1.37**	0.219	0.537	0.95	2.20
	M	**1.40[a]**	0.420	0.593	0.98	1.82
	F	**1.01[a]**	0.047	0.081	0.95	1.10
Serum urea (mg/dL)	M+F	**41.03**	3.084	7.555	29.70	49.70
	M	**37.80[a]**	1.100	1.555	36.70	38.90
	F	**46.97[a]**	2.161	3.743	42.70	49.70
Serum total protein (g/dL)	M+F	**7.52**	0.662	1.480	5.1	8.9
	M	**6.8[a]**	0.574	1.700	5.1	8.5
	F	**8[a]**	0.705	0.648	7.4	8.9

[a-a] p>0.05

Analyzing the previous table values, it was found that, in this age category, males registered a maximum creatinine value, higher than the physiological maximum limit (1.82 mg/dl), but the average value of this parameter did not exceed the maximum value. Also in this context, in the same age category, but also in both sexes, serum urea registered an average value higher than the species, respectively 37.8 mg/dl in males and 46.97 mg/dl in females.

Considering the above, we can say that FLUTD can affect the renal parenchyma in time. Regarding these two parameters, no significant differences were found at p>0.05.

Regarding the total serum proteins, it was not found, statistically, an average value of this blood biochemical indicator that does not fall within the physiological limits of the species (5.6-8.6 g/dl).

The following tables (Table 2, Table 3) show the average and the dispersion indices of the same parameters mentioned above, taking into account the cats from the experimental group, male and female, between 5 and 8 years old, respectively 9 and 11 years old.

Table 2

Average and dispersion indices of some blood biochemical parameters in cats between the ages of 5 and 8 years

Specification		Average	Medium error of the average	Standard deviation	The value	
					minimum	maximum
Creatinine (mg/dL)	M+F	**1.67**	0.159	0.357	1.35	2.20
	M	**1.72**	0.245	0.425	1.40	2.20
	F	**1.61**	0.200	0.368	1.35	1.87
Serum urea (mg/dL)	M+F	**38.54**	2.578	5.766	29.70	45.70
	M	**37.77**	4.619	8.001	29.70	45.70
	F	**39.70**	0.500	0.707	39.20	40.20
Serum total protein (g/dL)	M+F	**6.46**	0.852	1.907	5.4	8.0
	M	**6.47**	1.105	2.02	3.8	8.0
	F	**6.45**	0.755	1.05	5.4	7.5

$^{a-a}$ p>0.05

Analyzing the recorded values it is observed that with age, the average serum creatinine value of the two age categories exceeded the maximum physiological limit in males and females, only in the case of those between 9 and 11 years, 2.2 mg/dl.

The average value of serum urea in the two categories of cats in the experimental group showed higher values for the species, in both males and females, with the exception of females aged 9-11 years. This aspect can be explained by the fact that urea, being not a constant parameter, is dependent on water consumption and diuresis respectively.

Regarding the total serum proteins, their average value, from a statistical point of view, was within the physiological limits only in the category 5-8 years, in both sexes, and in the category 9-11 years they presented an average lower than the physiological limits demonstrating that in this situation there are urinary losses,

the diseases of the lower urinary tract being associated with a syndrome of renal insufficiency.

Table 3

Average and dispersion indices of some blood biochemical parameters in cats between the ages of 9 and 11 years

Specification		Average	Medium error of the average	Standard deviation	The value	
					minimum	Maximum
Creatinine (mg/dL)	M+F	**2.05**	0.080	0.179	1.82	2.30
	M	**1.95**	0.077	0.135	1.82	2.09
	F	**2.20**	0.100	0.141	2.10	2.30
Serum urea (mg/dL)	M+F	**35.14**	6.151	13.754	14.50	50.10
	M	**38.83**	5.750	9.960	31.20	50.10
	F	**29.60**	15.100	21.354	14.50	44.70
Serum total protein (g/dL)	M+F	**4.34**	0.266	0.594	3.7	5.1
	M	**4.7**	0.520	0.374	4.2	5.1
	F	**3.8**	0.418	0.122	3.7	3.9

[a-a] p>0.05;

Taking into account all the parameters mentioned above, there were no statistically significant differences at p>0.05.

Figure 1 shows the graphical representation of the blood biochemical parameters according to the age of the cats in the experimental group (Fig. 1).

From these we can see an upward aspect regarding serum creatinine, especially in cats aged 9-11 years. Serum urea registered a higher value in young cats (2-4 years), but in all three age categories, it had a value higher than the physiological maximum limit, probably secondary to the low water intake and stress which creates disorders in the diet and water consumption or urinary complications.

One of the dominant symptoms in cats in the experimental group was dysuria, even anuria, and from a behavioral point of view, they showed restlessness, frequent vocalizations, urination in the form of drops and dysorexia.

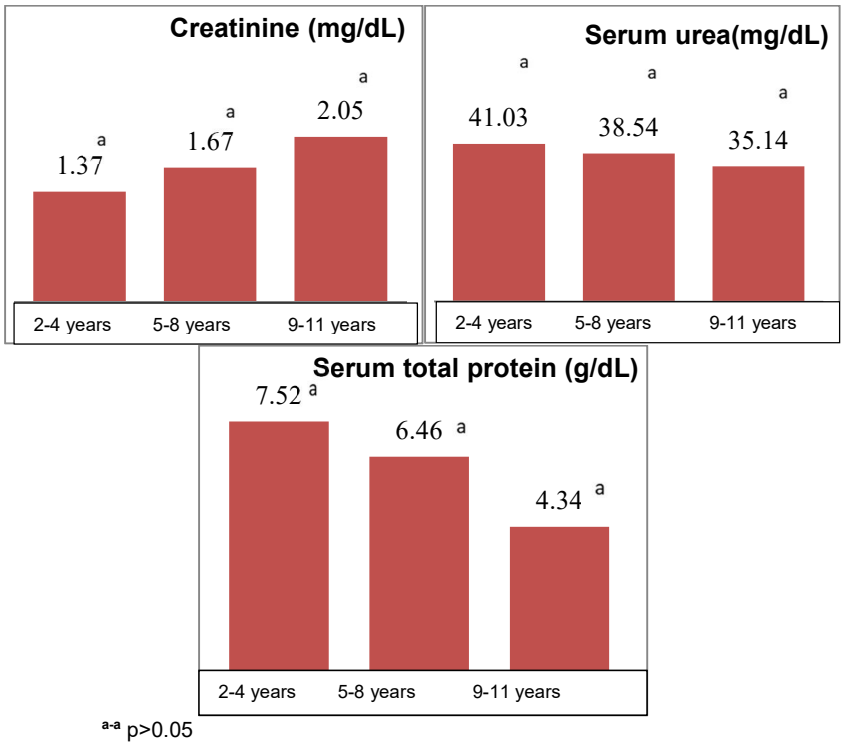

Fig. 1. Graphical representation of blood parameters in cats

The clinical examination did not reveal changes in volume in the bladder, but the ultrasound in B mode revealed an aspect of semiplenitude of the bladder, partially transonic, with an anecogenic aspect combined with areas of increased echogenicity, represented by the organic unorganized bladder sediment (Fig. 2).

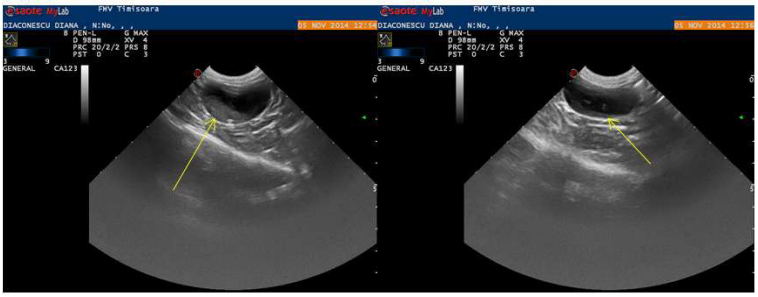

Fig. 2. Bladder ultrasound, B mode, longitudinal section (original)

The wall of the bladder had no ultrasound changes, a characteristic element for supporting the diagnosis of disease of the lower urinary tract.

Also, ultrasound changes were observed at the level of the urethra, this being slightly dilated, with ultrasound visibility and edematous walls (Fig. 3).

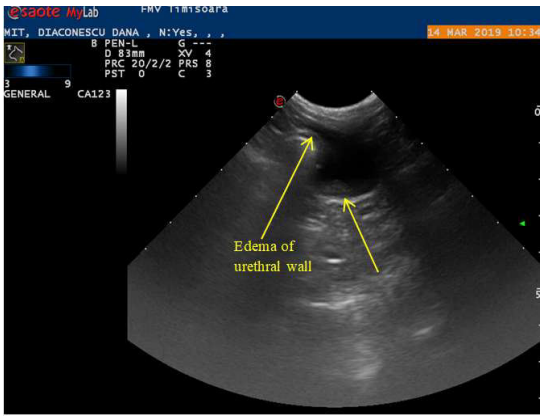

Fig. 3. Urinary bladder ultrasound with urinary sediment and urethral parietal edema, mode B, longitudinal section (original)

Also in this context, by abdominal ultrasound in cats in the experimental group, in a male and a female cat, which had an external symptomatology characterized by psychomotor agitation, repeated urination tests without completion, sometimes with the elimination of drops. Red urine, from an ultrasound point of view, was identified a high volume urinary bladder, evident hyperecogenic wall, edema line between serous and mucous membranes, and an inhomogeneous, anecogenic and hypo / hyperecogenic content (Fig. 4). This image is eloquent for the bladder block with bladder sediment in small quantities.

Also in the cats from the study of the diseases of the lower urinary tract, there were symptoms consisting of frequent urination, vocalization during urination, urination in different places, inconsistent hematuria, increased water consumption, subfebrility and blurred urine.

The ultrasound in B mode in these cats showed a bladder in semiplenitude, transonic, with anecogenic content, but the wall was thickened, the mucosa nonuniform and frequently line of interstitial edema, sero-mucosal (Fig. 5).

This edema from the level of the mucosa of the bladder extends to the level of the sphincter and the urethra, the latter having a thickened wall, with hypertrophy of the mucous layer and, finally, urethral stenosis (Fig. 6).

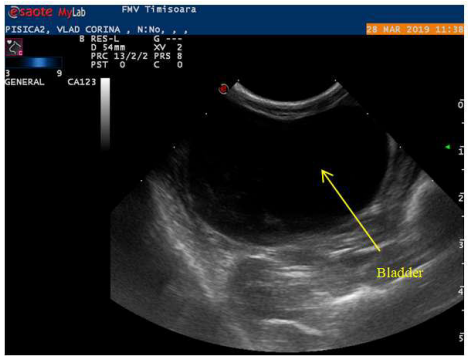

Fig. 4. Bladder ultrasound with bladder block, mode B, longitudinal section (original)

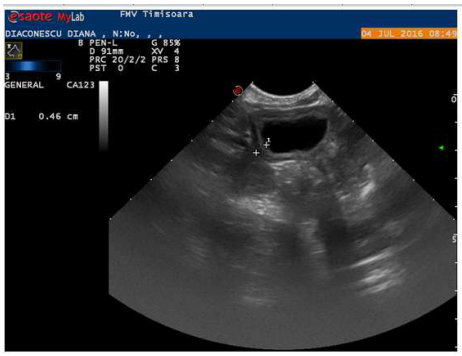

Fig. 5. Urinary bladder ultrasound with cyst, mode B, longitudinal section (original)

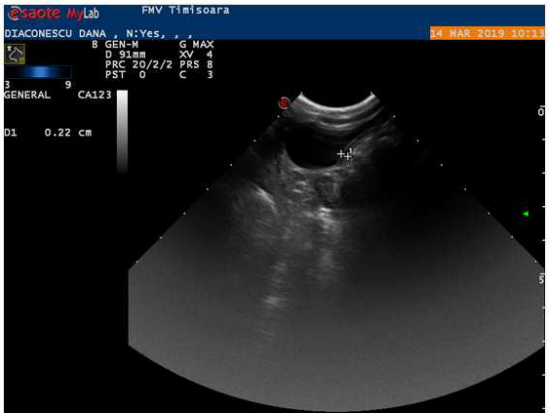

Fig. 6. Urinary bladder ultrasound with urethral stenosis, mode B, longitudinal section (original)

Conclusions

The biochemical parameters represented by creatinine, serum urea and total serum proteins are essential in the diagnosis and staging of renal failure syndrome, different from the lower urinary tract disease.

We recommend in cats with symptoms of lower urinary tract disease, performing abdominal ultrasound in B mode and color Doppler, to detect changes in the urinary tract.

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