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

FACULTATEA DE MEDICINĂ VETERINARĂ

LUCRĂRI ŞTIINŢIFICE

**MEDICINĂ VETERINARĂ
TIMIŞOARA
VOLUMUL LV (1)**

**SCIENTIFICAL PAPERS
VETERINARY MEDICINE**

To be cited: LUCRARI STIINTIFICE: MEDICINA VETERINARA TIMISOARA (SCIENTIFICAL PAPERS: VETERINARY MEDICINE TIMISOARA)**,** vol. LV (1), 2022

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

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2022**

PRELIMINARY RESULTS REGARDING THE MOLECULAR DIAGNOSIS OF CANINE DEGENERATIVE MYELOPATHY IN CARPATHIAN SHEPHERD DOG BREED

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Summary

Canine degenerative myelopathy (CDM) is an autosomal recessive disease characterized by progressive ascending degeneration of the spinal cord. The clinical features of CDM include progressive, asymmetric upper motor neuron paraparesis and lack of paraspinal pain. According to the literature, there are two mutations associated with CDM: a transition (*c.118G>A*) in the exon 2 of *SOD1* gene that has been described in several breeds, and a transversion (*c.52A>T*) in exon 1 of the same gene that has been described in Bernese Mountain dogs (2, 17). This study aimed to identify the proper technique for the identification of the mutation *SOD1:c.118G>A* associated with CDM, and to apply it on samples from Carpathian Shepherd dogs. Oral swabs were collected from 19 Carpathian Shepherd Dog and tested using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The results would classify the dogs as homozygous individuals for the normal allele (GG), heterozygous individuals (AG) or homozygous dogs with the mutation associated with CDM (AA). All of the dogs included in the study were identified as homozygous for the normal allele. Currently, there is no standard test for the identification of CDM. The diagnosis is mostly based on the exclusion of other diseases with similar symptomatology, and the definitive diagnosis is made following the postmortem histopathologic examination of the spinal cord. Therefore, DNA tests may be used to aid the presumptive diagnoses and for screening of CDM. Our preliminary results of the PCR-RFLP testing further recommends this technique as a mean to identify heterozygous carriers (AG) and establishing the affected individuals (AA).

Keywords: canine degenerative myelopathy, Carpathian Shepherd dog, PCR-RFLP, SOD1.

Canine degenerative myelopathy (CDM) is a fatal neurodegenerative disease which affects adult dogs of several breeds. The onset of the of the clinical signs is around the age of eight, and includes symptoms such as progressive, asymmetric upper motor neuron paraparesis and general proprioceptive ataxia of the pelvic limbs, progressing to paraplegia and eventually flaccid tetraparesis (6). The disease was first described in German Shepherd dog breed (1).

The precision of the antemortem diagnosis is based on the identification of the progressing clinical signs, followed by the exclusion of other diseases. Neurological diagnostic of the diseases affecting the spinal cord include analysis of the cerebrospinal fluid, electrodiagnostic tests and medical imaging procedures such

as myelography and MRI (6, 10).

Postmortem histologic examination highlights primary central axonopathy of the spinal cord. The associated axons and myelin degenerates following a segmented pattern which affects all funiculi and involves mainly proprioception, motor and sensitive neurons. No degenerations or losses are observed in the neuronal perikarya (4, 13). The diagnosis of CDM is not facile, as similar clinical signs can be observed in other neurological diseases such as lumbosacral stenosis or intervertebral disk disease. Antemortem diagnosis of CDM is often based on the exclusion of other neurological diseases, as there is no defined method of diagnostic (6).

From a genetic point of view, CDM is an autosomal recessive disease with incomplete penetrance and two causal mutations described. The first mutation was reported in Boxer, Rhodesian Ridgeback, German Shepherd, Pembroke Welsh Corgi and Chesapake Bay Retrievers, but is not limited to these breeds. It is characterized by a G to A transition (*c.118G>A; p.E40K*) in exon 2 of *SOD1*, which leads to a change from glutamate to lysine in amino acid 40 from the polypeptide chain (2). The second mutation was reported in Bernese Mountain Dog and is a *c.52A > T* transversion in exon 1 of the same gene (*SOD1*) which leads to a change from threonine to serine in amino acid 18 of the polypeptide chain (17). In Pembroke Welsh Corgi, a haplotype has been described as a variation factor which modifies the onset period of CDM. Variations in the genetic transcription of SP110 haplotype (nuclear body protein) observed in this dog breed may be partially associated with the risk of developing CDM at a younger age (9).

Carpathian Shepherd dog breed was accepted on a definitive basis by the Fédération Cynologique Internationale (FCI) on 9th of June 2015. The first standard was written in 1934 by the Zootechnical National Institute from Romania. The Carpathian Shepherd dog was selected from an endemic dog breed from the Carpathian-Danubian geographical area, with focus on its utility (19). Currently, there is a single scientific study which involved the Carpathian Shepherd dog and described the molecular characterization of Romanian shepherd dogs (5). Additionally, the PhD thesis of the same author elaborated a phylogenetic tree of the Carpathian Shepherd dog which identified the following related dog breeds based on the mitochondrial DNA: Tibetan Mastiff, Saint Bernard, Great Dane and Schnauzer (14).

The aim of the current study was to identify the proper technique for the identification of the mutation *SOD1:c.118G>A* associated with CDM, and to apply it on samples from Carpathian Shepherd Dog.

Materials and methods

1. Selection of individuals and sampling

Individuals which belong to Carpathian Shepherd dog breed and confirmed by pedigree documentation were selected for inclusion in this study. 19 dogs were included, from which 9 were females and 10 were males, with ages ranging from 1

to 10 years (Table 1). All the individuals included in the study were healthy when the sampling was done.

The samples were collected from a kennel in Bistrița-Năsăud County, Romania. Sterile, cotton buccal swabs were used for sample collection. The swabs were rubbed against cheek mucosa for 10 seconds on each side of the mouth. The collected samples were transported at 4°C and stored at −20°C.

Table 1

Gender and age distribution among the dogs included in the study

No.	Date of birth	Gender
1.	13.08.2016	F
2.	29.06.2020	M
3.	06.05.2018	F
4.	01.05.2018	M
5.	05.12.2014	M
6.	26.06.2018	M
7.	13.03.2017	F
8.	12.10.2017	M
9.	12.10.2017	F
10.	03.05.2016	F
11.	01.12.2017	M
12.	15.12.2012	F
13.	09.03.2013	M
14.	02.05.2011	M
15.	09.03.2013	M
16	17.05.2018	F
17.	28.09.2016	F
18.	29.06.2020	F
19.	22.06.2013	M

2. DNA extraction, isolation, amplification and identification of region SOD1

The extraction and isolation of the DNA from the collected samples was done with Isolate II Genomic DNA Kit (Bioline) in accordance to the manufacturer protocol. The isolated DNA was amplified by polymerase chain reaction (PCR) in order to amplify the region of canine gene *SOD1*. The following primers were used: forward (DF) - 5′-AGTGGGCCTGTTGTGGTATC-3′ and reverse (DR) - 5′-TCTTCCCTTTCCTTTCCACA-3′. The primers were diluted in accordance to the manufacturer instructions. The PCR was done using 25 µl reaction volumes. The PCR mix included: 12,5 µl MyTaq Red Mix (Bioline), 1 µl forward primer, 1 µl reverse primer, 4 µl ADN și 6,5 µl ultrapure H_2O. In order to establish the proper amplification temperature, 5 samples were amplified in TermoCycler C1000TM (Bio-Rad Laboratories) by using two incubation protocols as per Table 2. Both protocols were

adapted to the instructions recommended by the manufacturer of MyTaq Red Mix.

The amplification products were detected after electrophoreses in 2% agarose gel and examined in UV transilluminator BioDoc-It (Bio-Rad Laboratories). The expected PCR result for *SOD1* gene was an amplicon of 292 base pairs (bp).

Table 2

Incubation protocols used for PCR

Protocol I			**Protocol II**		
Cycles	Temperature	Time	Cycles	Temperature	Time
	95°C	5 min		95°C	5 min
40 x	94°C	30 sec	40 x	94°C	40 sec
	53.2°C	30 sec		55°C	30 sec
	72°C	1 min		72°C	1 min
	72°C	5 min		72°C	5 min

3. Investigation of the presence of mutation *C.118G>A* by restriction fragment length polymorphism (RFLP)

The amplified DNA samples were recovered using Gel/PCR DNA Fragments Extraction Kit (Geneaid), in accordance to the manufacturer protocol. The resulted products were digested using AcuI enzyme using the following mix: 2.5 µl digestion buffer, 11.5 µl ultrapure H_2O, 1 µl AcuI enzyme and 10 µl PCR product. The samples were incubated at 37°C for 6 hours, followed by inactivation at 65°C for 20 minutes.

In order to determine the genotype of the dogs included in the study, the DNA samples resulted following the digestion were migrated in electrophoreses in 3% agarose gel. The results would classify the individuals as homozygous with normal allele (GG) with amplicons of 62 and 230 bp, heterozygous (AG) with amplicons of 62, 230 and 292 bp, and homozygous with mutant allele (AA) associated with CDM with amplicon of 292 bp.

Results and discussions

Samples 1–5 were incubated according to the protocols mentioned in Table 2. Protocol I was referenced from a research conducted by Santos et al. (15) in which the authors surveyed the presence of the CDM associated mutation in German Shepherd dogs in Brazil (15). Protocol II was referenced from a research conducted by Holder et al. (8) in which the authors assessed the prevalence of CDM associated mutation in German Shepherd dogs in United Kingdom (8). Both protocols offered adequate results, with 292 bp amplicons for all 5 samples (Fig. 1).

Sample 4 was selected from both protocols for further testing of RFLP reaction, as the sample was the one best highlighted. The results following RFLP are shown in Fig. 2. Due to the better highlighted amplicons resulted using Protocol II, the 19 samples were tested using this protocol.

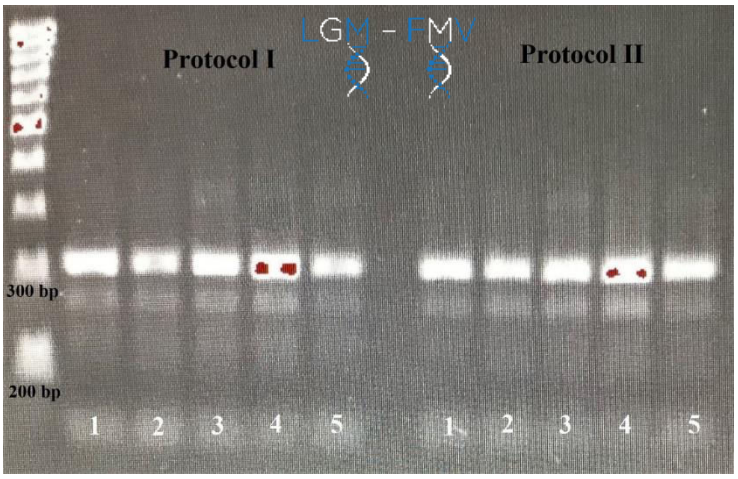

Fig. 1. PCR amplification results using the protocols mentioned in Table 2

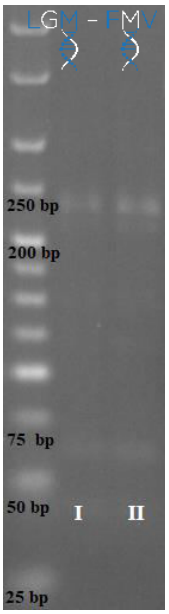

Fig. 2. RFLP results for sample 4 using Protocol I and II

The results of the PCR amplification for samples 1–5 are presented in Fig. 3 and the results for 6–19 are presented in Fig. 4. All 19 samples highlighted

amplicons of 292 bp corresponding to the canine *SOD1* gene. A 100 bp molecular weight ladder was used to determine product sizes (HyperLadder 100bp, Bioline).

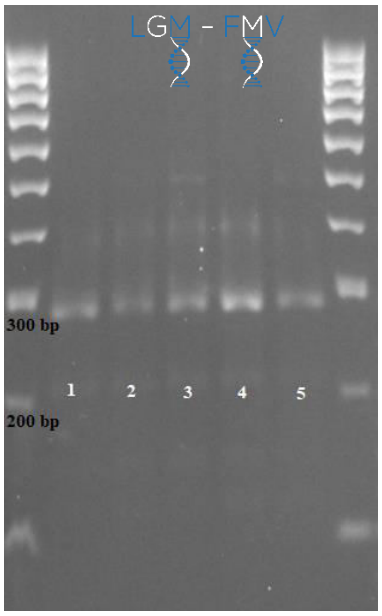

Fig. 3. PCR amplification results for samples 1–5

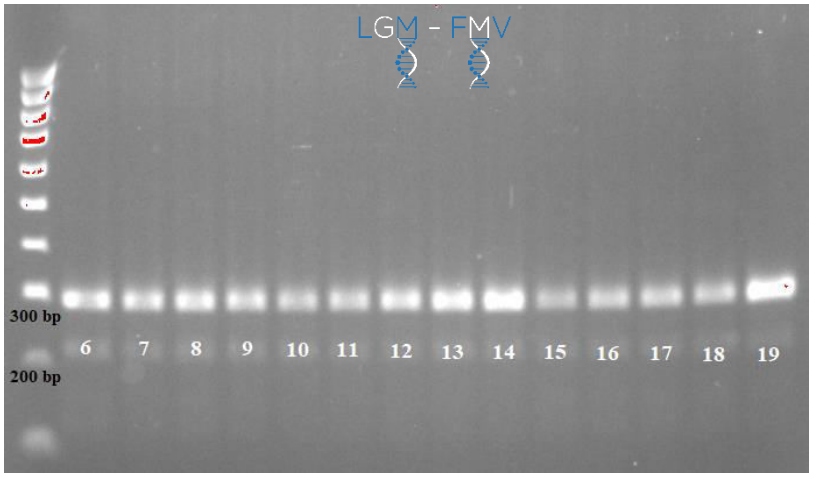

Fig. 4. PCR amplification results for samples 6–19

The results of the RFLP genotyping for samples 1–12 are presented in Fig. 5 and the results for 13–19 are presented in Fig. 6. A 100 bp molecular weight ladder was used in the first and the 15th column (HyperLadder 100bp, Bioline), and a 25 bp molecular weight ladder was used in the second column (HyperLadder V, Bioline).

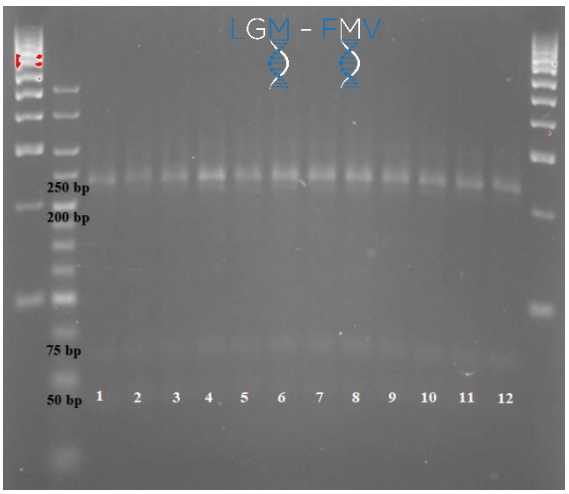

Fig. 5. RFLP genotyping results for samples 1–12

All of the 19 Carpathian Shepherd dogs included in the study were classified as homozygous with normal allele (GG) according to the 62 and 230 bp highlighted amplicons from Fig. 5 and Fig. 6. PCR-RFLP genotyping was proven as an efficient method of molecular surveillance for CDM in Carpathian Shepherd dog.

The mutation *c.118G>A* associated with CDM was initially identified in a genome-wide association study and was characterized through manual sequencing (2). Canine *SOD1* gene genotyping is considered as a useful tool in the diagnosis of CDM. Heterozygous individuals may be prone to developing concomitant pathological processes. CDM management is problematic due to the rapid progress of the clinical signs. Hence, the result of a genetic test for an individual who is homozygous for *SOD1:c.118G>A* mutation may influence the treatment that it receives by excluding the surgical procedures of comorbidities associated with the spinal cord and emphasizing on physiotherapy which was proven to be a palliative approach for dogs diagnosed with CDM (11).

Epidemiological data regarding the genetic diseases characteristic to each dog breed is essential in establishing prevention measures (9). Epidemiological surveillance should be conducted in every country and region, and the results should be known worldwide so that the distribution of the disease specific allele can be mapped (12).

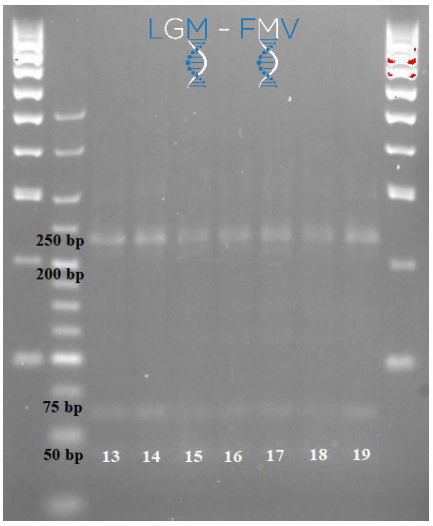

Fig. 6. RFLP genotyping results for samples 13–19

Knowledge of the allele distribution frequency for *SOD1:c.118G>A* mutation in dog breeds prone to developing CDM can be useful in choosing better strategies for selective breeding (2). A relatively high prevalence (estimated at 0.35) of the mutant allele for CDM was reported in German Shepherd dog, which may be a problem when selecting individuals for breeding purposes (8). Additionally, in a study conducted on 72 purebreed Belgian Malinois dogs, the genotype frequency for the heterozygous (AG) individuals was 25%, while the frequency for the homozygous mutant (AA) allele was 4% (15). An even greater prevalence of mutant alleles (>0.7) was reported in Boxers and Pembroke Welsh Corgis (18). Radical restriction of breeding among mutant allele carrier individuals may lead to undesirable consequences such as loss of desired characteristics or the unintentional selection of other mutations (8).

Similarities have been noticed between canine degenerative myelopathy and amyotrophic lateral sclerosis (ALS) in humans. Of these, the most notable are oligodendrocyte injury which leads to demyelination, upregulation of CB2 receptors in reactive astrocytes and increase of arginase 1-expressing macroglia in the proximity of motor neurons (4). The onset of ALS is between 45 and 60 years, when the degenerative processes of the upper and lower motor neurons peaks with paralysis and death (3). Genetic studies of familial ALS have identified an association between the disease and the mutation of the gene that codes superoxide dismutase (*SOD1*). *SOD1* is an enzyme involved in the elimination of superoxide free radicals from cells and, furthermore, has an important role in reducing oxidative stress and damage. Studies suggest that the enzyme activity is not influenced by the presence of the *SOD1* mutation, but the proteins coded by the mutant variant have proven a

tendency towards forming aggregates. A hypothesis regarding the progressive manner of CDM states that *SOD1* aggregates may propagate from cell to cell. Mutant SOD1 proteins are released from cells and further induce aggregate formation of the normally folded *SOD1* proteins in the cells by which they are taken (18). However, the relation between this mechanism and the pathogenesis of neuronal dysfunctions observed in CDM and ALS has not yet been established (7, 16).

Conclusions

The current study is the first one which concerns the molecular surveillance of the mutation *SOD1:c.118G>A* associated with CDM in Carpathian Shepherd dog. PCR-RFLP was proven as an easy, fast and specific method for this purpose. As there is no standard antemortem test for the identification of CDM in dogs, this genetic test offers the option to practitioners to identify the carriers or affected individuals by the *SOD1:c.118G>A mutation* at early stages and to establish a differential diagnosis.

Knowing the genotype of a young individual with a known mutation may be beneficial for the future clinical decisions. Homozygous dogs (AA) for *SOD1:c.118A* mutation may be presumptively diagnosed with CDM, but it is important not to neglect concomitant diseases that may arise. The use of PCR-RFLP testing for the identification of *SOD1:c.118A* mutation associated with CDM was proven as an efficient and specific mean of diagnosis.

Identification of carriers and affected individuals at early stages can be beneficial in the establishing the future clinical approach by the practitioner. On this regard, the PCR-RFLP testing is an suitable option for the early diagnosis of CDM.

The usage of genetic testing may be important for the improvement of breeding programs in dog breeds that are known to be predisposed to CDM. Thus, rational decision should be made on this matter in order to maintain the desired traits.

The main means of genetic prophylaxis consists of removing the carrier individuals from the breeding population. Although it is a fast way to reduce the frequency of the mutant allele in the population, this may lead to the reduction of the genetic pool of the concerned dog breed. Heterozygous individuals may be used for breeding but only with non-carriers. The puppies should be tested as 50% of them would be carriers of the mutant allele.

Acknowledgement

The authors are grateful to the owners of the dogs involved in the study for their willingness to collaborate.

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RESULTS REGARDING THE USE OF THERAPEUTIC PROTOCOLS IN ROMANIAN SPOTTED COW WITH REPRODUCTIVE DISORDERS

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Summary

Ovarian dysfunction, repeated breeding and postpartum endometritis are the most common reproductive disorders in cattle breeding because they lead to a large economic loss for the farmer due to more inseminations, an increase in in calving interval, an increase dry periods length, increase in veterinary examination and treatment costs. Hormonal therapy is used as a treatment for repeat breeding, ovarian dysfunction or to ensure pospartum reproductive health. In our study performed on 35 cattle raised in the traditional system, we evaluate the results of hormonal therapy in repeat breedings, ovarian chists, and endometritis. Of these cows with an average lactation of 3.91; 20 had ovarian dysfunction such as follicular cyst or luteinic cyst (group A1), 9 cows had repeated breeding (group A2) and 6 cows had endometritis (group A3). The avearge insemination rate in this study was 4.4. The treatments applied were the following: in group A1 GnRH followed at 7 days by PGF2α (for lutein cyst) and HCG twice at 0 and 6 days followed after 7 days by PGF2α (for follicular cyst), in group A2 progesterone and multivitamins were administered on days 4 and 7 after artificial insemination and in group A3 antibiotics and intramuscular anti-inflammatory drugs were used. The cows were artificially inseminated at the first signs of heat after treatment. The rate of conception after the therapeutic protocols was 95% for group A1, 100% for group A2 and 33.33% for group A3. In conclusion, the data of this study highlight the differences in therapeutic protocols applied in some reproductive diseases in cattle, suggesting the diversity of factors that can induce these disorders.

Keywords: cattle, reproductive disorders, ovarian cyst, endometritis.

Low input farms and Romania dairy family farms are still on the milk market and they are under the pressure of economic efficiency and as well as under the reproductive physiological rules (7). The most common reproductive disorders in cattle are ovarian dysfunction, repeat breeding and postpartum endometritis, for both intensive and traditional breeders, disorders that lead to large economic loses (Fig.1)(21).

Reproductive performance is one of the most important factors affecting cattle farm profitability and the living standard of rural and urban societies, because, influences directly or indirectly the yield of milk, reproductive culling rate and the cost for breeding and calf sales. Dairy cows should calve one time every year to maximize economic efficiency (1).

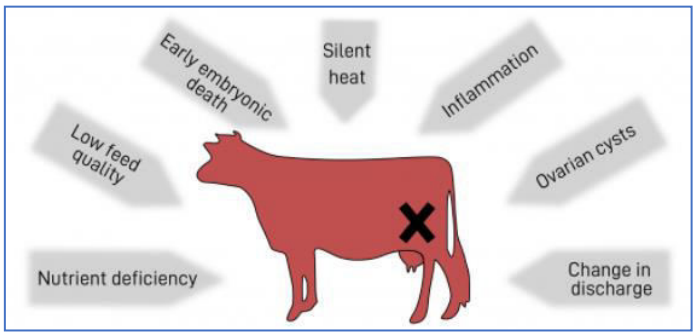

Fig. 1. Factors that can affect reproductive performance in cows (21)

Materials and methods

The study was carried out on 35 Romanian Spotted cows raised in the traditional system, that were divided into 3 groups based on their reproductive problem as follows: group A1 - 20 cows with ovarian dysfunction such as follicular cyst or luteinic, group A2 - 9 cows with repeated breeding and group A3 - 6 cows with endometritis. The treatments applied were the following: in group A1 GnRH followed at 7 days by PGF2α (for luteinic cyst) and HCG twice 0 and 6 days followed after 7 days by PGF2α (for follicular cyst), in group A2 progesterone and multivitamins were administered in days 4 and 7 after artificial insemination and in group A3 intrauterine antibiotics and intramuscular anti-inflammatory drugs were used (Table 1).

Table 1

Drugs administrated in this research

Group of animals	*Specimen*	*The active substance*
A1 group - luteinic cyst	Receptal®	Buserelin acetate
	Proliz®	Cloprostenol
A1 group - follicular cyst	Chorulon®	Human Chorionic gonadotropin (hCG)
	Proliz®	Cloprostenol
A2 group	Romprogestin® Vitol-140®	Progesterone Vitamin E, A, D3
A3 group	Metrosept®	Enrofloxacin, oxytetracycline chlorhidrate, iodoform

Results and discussions

The results obtained are represented graphically in Fig. 3-5. Fig. 3 shows the number of cows from each group that responded to the specific treatment. In group A2, cows with repeat breeding, the administration of progesterone (P4) 4 and 7 days after artificial insemination was 100% effective, which may indicate that

the recurrence of heat was probably caused by embryonic mortality. Repeat breeding occurs when ovaries produce low-quality oocytes and embryos, when the uterus is unable to sustain pregnancy due to hormonal insufficiency and dysfunction. The last cause contributes about 40.1% of the causes of repeat breeding (20). Other causes can be the age, repeated breeding has a higher incidence in older cows, the nutrition, milk yield, lactation number or dystocia (1).

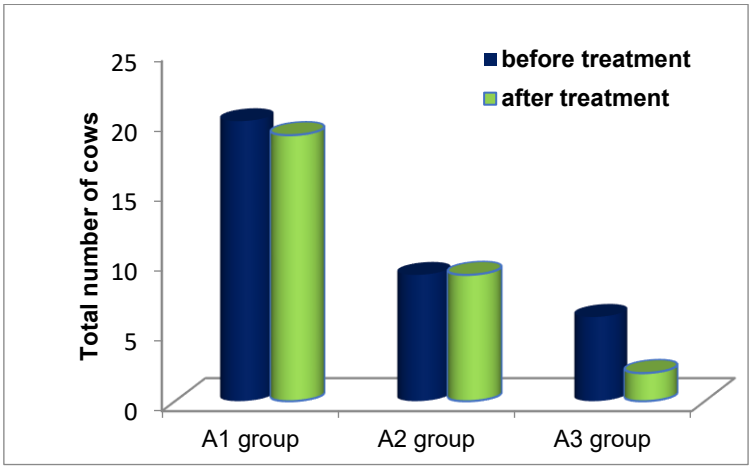

Fig. 3. Total number of cows before and after hormonal treatment

Similar results were seen in our research, where the cows in group A2 were on average in the 5th lactation, compared to group A1 where the parity average was 2.83 or to group A3 where it was 3.63. In group A1 with the cows that had ovarian cysts, they respond well to the hormonal treatment, with a 98% rate of conception. Ovarien cysts are dynamic structures, and their development and lifespan are likely associated with altered hypothalamic-hypophysial-ovarian function, with lower hypothalamic content of GnRH compared to healthy cows (5). Ovarian cyst are defined as cystic ovarian follicular structures of at least 17 mm that persist for morethan 6 days in the absence of corpus luteum. More recently, the ovarian cystes are defined as anovulatory ovarian structure greater than 20 mm in diameter and no corpus lutem (2). Follicular cysts are anovulatory structures associated with low concentrations of progesterone in the peripheral circulation with suboptimal luteinizing hormone (LH) surge and persistently increased estradiol levels, while luteal cysts are associated with high concentrations of progesterone, are smooth and round, and their walls are thicker compared to follicular cysts. Luteal cysts have a spherical cavity lined by a layer of fibrous tissue surrounded by luteinized cells (1, 8). Another difference between the follicular cyst and the luteal cyst is the thickness of the wall, a thickness that can be measured by ultrasound,

and if it is less than 3 mm it is the follicular cyst and if it is larger than 3 mm it is a luteal cyst (3). The concentration of progesterone in the peripheral circulation is different, so in follicular cyst is below 1 ng / ml, and in lutein cysts the concentration is higher than 1 ng / ml (8).

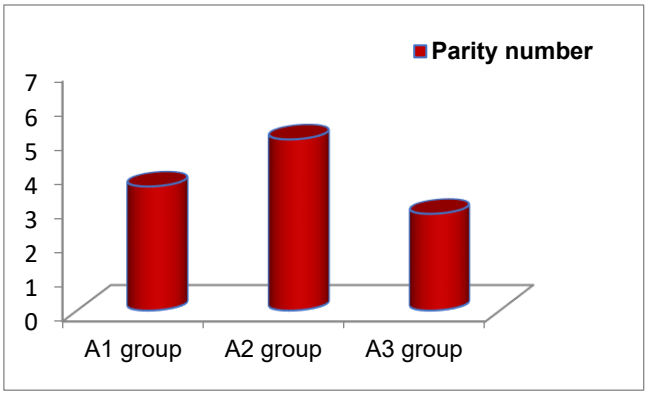

Fig. 4. Average no of parity /groups

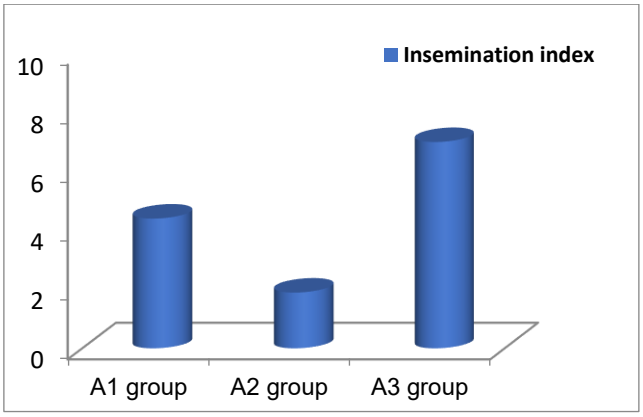

Fig. 5. Insemination index/groups

The ovarien cysts theraphy is very numerous and variable, with many endocrine based treatments for cysts (6, 12, 18). Data from the literature indicate that hormonal treatment with Gn-RH and PGF2alpha in ovarian cysts has better results in terms of conception and gestation rate after treatment compared to treatment with Gn-RH alone (14). For lutein cysts, treatment with prostaglandin in the luteolytic dose is indicated, followed by a 75% rate of heat entry and a 66%

rate of gestation (9). Chebel et al. (4) showed that in herds in which a rigorous and accurate detection of estrus was made, presynchronization with PGF2alfa can reduce the time to the gestation. The application of GnRH and PGF2alpha-based TAI (fixed-time artificial insemination) protocols to synchronize the appearance of a new follicular wave, luteal body regression, and ovulation showed that cows that ovulated after the first administration of GnRH were more likely to have synchronized ovulation at the end of the protocol and an increased gestation rate at the first AI (4). As such, ovulation produced after the first administration of GnRH improves fertility both by synchronizing ovulation near the time of artificial insemination and by reducing the period of follicular dominance (18, 19). As the ovulatory response to GnRH injection also depends on the release of pituitary LH - affected by circulating progesterone, an alternative to prevent the inhibitory effect of P4 on ovulation may be the use of hormonal preparations capable of inducing ovulation when progesterone levels are elevated. One such hormone is human chorionic gonadotropin (hCG) which is capable of inducing ovulation in a highly progesterone environment as hCG triggers ovulation by direct coupling to LH follicular receptors. Another variant is to increase the dose of GnRH in order to prevent the inhibitory effect of P4 on the pituitary release of LH and intensification of the ovulatory response (6).

The rate of conception after the therapeutic protocols were 95% for group A1, 100% for group A2 and 33.33% for group A3. For traditional breeders, for cows in which the nutritional intake that does not meet the higher energy requirements can cause a negative energy balance and this negative energy balance during the early postpartum period, a weaker body condition causes an energy deficiency for hormone synthesis and secretion to ovulate a follicle and maintain an early developing embryo (16). The average number of parity (Fig. 4) in group A3 was 2.83, the lowest compared to the other groups, but with the weakest responses to treatment, compared to group A2, where the average is 5 and A1 where the average is 3.63, which may be an indication of a reduced acquired immunity.

Endometritis is another reproductive disorder very common, affecting up to 40% of post-calving cows. Endometritis is characterized by an inflammation of the endometrium more than 21 days post-partum and not associated with systemic signs of illness (1, 10). Clinical endometritis is characterized by the presence of purulent or mucopurulent discharge and a cervical diameter > 7.5 cm at ≥21-26 days post -partum without general symptoms. The success rates of treatment depends on severity of the case, in mild cases the success are higher compared to severe ones. Broad spectrum antibiotics, cephalosporins and oxytetracycline are effective in the uterine environment and are considered as the drugs of choice, in some cases prostaglandin (PGF) injections may be helpful (1).

This pathology found in cows from group 3 determined the higher number of AI compared to the other two groups, and the the lowest recovery rate by gestation after treatment, only 33.3%. Referring to the number of lactations, in this group, group A3 the average number of lactations was the lowest, 2.83. Analyzing

these data, it can be seen that in this disorder the response to treatment was the lowest, and the affected cows were young, probably due to an immune system less exposed to pathogens. Molecular and hormonal changes are complex in endometritis, so the synthesis of PGE2 and PGF2α are affected, which indirectly affects luteal function, and cytokines, mediators of inflammation, in large quantities will have a disturbed ovarian regulatory function (13).

Due to the risk of microorganism resistance to antibiotics, another possibility in the treatment of endometritis in cows is the use of immunomodulators, which initiate local defense mechanisms. Among the immunomodulators cited in literature data, we mention *E coli* lipopolysaccharide, glycogen oyster, filtered bacteria-free, serum, plasma or hyperimmune serum, levamisole, leukotrienes B_4, human recombinant interleukin-8 (10, 15, 17). Also, the use of phytotherapy alone or in combination with antibiotics has been tried with promising results (10). Nanotherapy is another way to reduce the number of endometritis in cattle. This therapy is based on the study of the possibilities of inhibiting the infectious signal at the level of cellular receptors during signal transduction process, interfering with the activation of transcription factors, pro-inflammatory cytokines and gene expression (11).

Conclusions

The data of this study highlight the differences in the therapeutic protocols applied in some reproductive diseases in cattle, suggesting the diversity of factors that can induce these disorders. The results show the relationship between the female age, the number of lactation and the parity rate.

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MORPHOLOGY OF THE SKULL IN BADGER (*MELES MELES*)

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Summary

Badger is a small stout carnivore spread in different parts of the world, including our country. Taxonomical studies of the carnivores do not include the descriptive and comparative anatomy of the skull. In the last decade, osteological features of some wild animals were studied within the Anatomy department from our faculty and many changes were recorded. No one of them involved badger.

Keywords: badger, skull, descriptive anatomy.

The European badger (*Meles meles*) is a stocky, powerful animal, belonging to the *Order Carnivora*, *suborder Caniformia, family Mustelidae* (18). Its distribution is various, being found in much of the USA, United Kingdom, Scandinavia, and rest of the Europe, but also in the far east as Japan and China (18). During mating period can cohabite with wild cat, fox and raccon dog (6, 12). It is considered the largest land predator in the Uk (19).

The characteristic aspect, black and white striped face, grey fur, and short furry tail make the badger an easily recognized creature (20).

Among the members of the mustelid group, the European badger (*Meles meles*) is larger than the American badger (*Taxidea taxus)*, while the two species of Asiatic badger belong to the genus *Mydaus* (15, 18, 19).

Even some people think badgers could be kept as pets, like other wild animals, they are not suitable as companion animals and suffer from the stress of captivity. Due to their temperament, these wild animals can inflict serious injuries. In addition, they are vectors for some diseases that could be dangerous to humans, spreading bovine tuberculosis to the cattle (19).

Knowledge the features of the skull in this animals are important when study the degree of similarity between species, mainly for taxonomic classification and differentiation (1, 2, 3, 4, 5). Therefore many studies were conducted in some species with risk of extension (14, 16).

Also, these observations represent interspecific and sexual differences in the skulls of some species (7, 8, 9, 10, 13).

In addition, anatomical relevance is given by the absence from the literature of specific data concerning the features of the skull in the European badger.

In this study were highlighted the skull particularities in the European badger, as well as offering data for breeders, professionals working in zoos,

veterinary practitioners or forensic medicine in relation to different poaching procedures when skull expertise is requested.

Materials and methods

For this study were used two skulls from the European badger (*Meles meles*). The animals were involved in a serious car incident and did not survive. The cadavers were transported to the Anatomy department, Faculty of Veterinary Medicine Timisoara and investigated.

Due to the accident the thoracic and abdominal cavities as well the thoracic limbs were completely damaged, while the head and the pelvic limbs showed less injuries.

The skulls were removed from the atlantooccipital joint undergoing the specific procedures for its preparation. The skin, muscles, blood vessels were dissected and removed, and the specimen was immersed in water and brought to the boiling point for six hours/day in four successive days.

Because the age of the animals could not been established the preparation was made with great attention, controlling every day the aspect of the skull and the degree of the flesh detachment. After four days the skulls were removed from the water, cleaned and perfused for two days with high pressure water and when it was considered ready were dried out naturally. Whitening the skulls was performed using a 15% peroxide solution for two hours/day during couple days.

Identification and description of bones were performed according to *Nomina Anatomica Veterinaria* 2017 (17) and lateral, dorsal, ventral and rostro-caudal photographs were made (Fig. 1).

Results and discussions

Analysis of the skull of European badger yielded a few distinctive particularities. The external surfaces of the bones of cranium and mandible have smooth appearance in some places, especially for the bones of face and over the frontal and parietal (11, 15).

Between the bones of neurocranium and viscerocranium there is a plane transition, no any sutures can be identified (Fig. 2).

In the ventral view, it was noticed that the occipital is very extensive, perpendicular to the longitudinal axis. The paracondylar processes are very short and the jugular notch is large.

The tympanic bulla is large, presenting triangular contour. The transition between the basioccipital and basisphenoid bones is equalized and the muscular tubercle is unique.

The choanae resemble a triangular contour.

The palatine bones are perforated by two major palatine foramina on each half, the hard palate has a rhomboidal contour, while the incisive part is perforated by two oval fissures.

The articular surface for the mandibular condyle is like a canal and bordered caudally by a bony ridge which is limiting the movement of the mandible.

In the dorsal view, the skull presents the characteristic of carnivores, showing an external sagittal crest long and well-outlined, extending rostrally to the frontal bones (Fig. 3). The interparietal bone is not presented.

Due to the absence of the zygomatic process of the frontal bone, the orbit is incomplete as in carnivores.

The nasal bone is long, smooth and rostrally ends abruptly.

The infraorbital foramen is very large, oval and located at the base of the temporal process of the zygomatic bone, dorsal to the first upper molar (Fig. 1).

Fig. 1. Rostro-caudal view of the skull of the badger (*Meles meles*)
1. External sagittal crest.; 2. Parietal bone; 3. Occipital bone; 4. Orbit; 5. Infraorbital foramen; 6. Maxilla; 7. Frontal bone; 8. Nasal bone; 9. Incisive bone.

The mandible is unpaired, fused bone. The shaft of the mandible extends caudally and forms a short angular process. The vascular notch is absent, while the mental foramen opens ventral to the first lower premolar. The head of the mandible is cylindrical, elongated. The coronoid process is triangular and the masseteric fossa is large and deep. The mandibular foramen opens near the angular process (Fig. 4, Fig. 5).

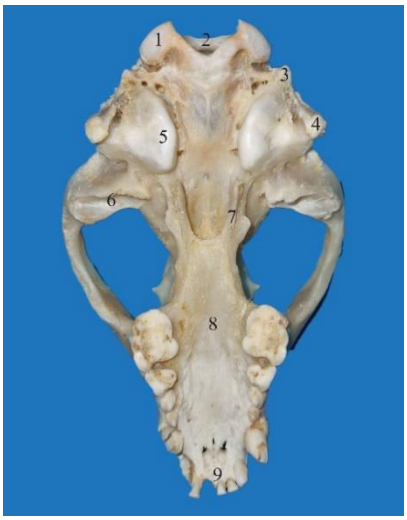

Fig. 2. Ventral view of the skull
1. Occipital condyles; 2. Foramen magnum; 3. Paracondylar process; 4. Mastoid process. 5. Tympanic bulla; 6. Mandibular fossa. 7. Pterygoid process; 8. Palatine bones; 9. Incisive bones

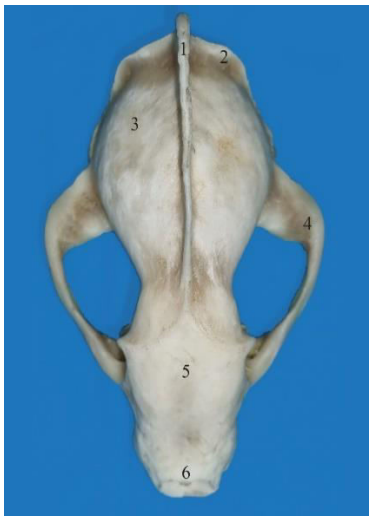

Fig. 3. Dorsal view of the skull
1. External sagittal crest; 2. Occipital bone; 3. Parietal bone; 4. Zygomatic arch; 5. Nasal bone; 6. Incisive bone

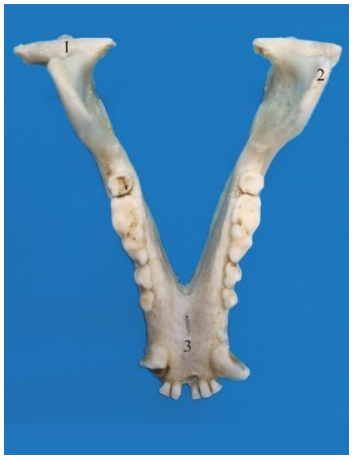

Fig. 4. Dorsal view of the mandible of the badger (*Meles meles*)
1. Head of the mandible; 2. Coronoid process; 3. Mandibular symphisis

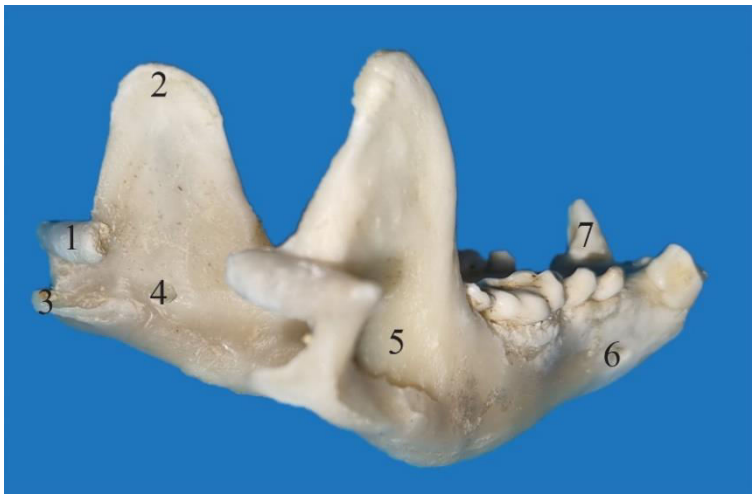

Fig. 5. Caudolateral view of the mandible in badger (*Meles meles*)
1. Head of the mandible; 2. Coronoid process; 3. Angular process; 4. Mandibular foramen; 5. Masseteric fossa; 6. Mental foramen; 7 Canine tooth

Conclusion

The external sagittal crest is long as in carnivores but broader, while the squamous part of the occipital bone is like a trough.

The paracondylar processes are very short and the jugular notch is large. The mastoid processes are long and orientated rostro-ventral.

The zygomatic arch is incomplete due to the absence of the zygomatic process of the frontal bone.

The frontal bone is rectangular in shape and the supraorbital foramen is not in place.

Nasal bones are very short.

The infraorbital foramen is large and oval.

The two mandibles form the symphysis, while the angular processes is long, restricting the degree of mouth opening.

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THE EFFECT OF TEMPERATURE ON *IN VITRO* BIOFILM FORMATION IN MULTIDRUG RESISTANT *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS*

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Summary

The crystal violet (CV) microplate assay is the most commonly used quantitative method to analyse biofilm-biomass formation, inhibition and eradication. This protocol allows variable incubation periods and temperatures, depending on the studied microorganism, but for microorganisms that do not require special temperature the value of 37°C is usually chosen. This study aimed to compare the biofilm formation through the optical density measurement (OD at 540 nm) of two multidrug resistant *E. coli* and *S. aureus* strains, at different temperatures: 22, 30 and 37°C (22.34 ± 0.12; 30.11 ± 0.42; 36.97 ± 0.13°C) for 48 h, in a controlled environment incubation unit (invention currently under review for patent) – a system designed to prevent the higher gas exchange and evaporation from the outer wells of the microplate. The temperature proved to be a significant factor in biofilm formation for both pure and mixed culture biofilms (*ANOVA* - $F_{E.\ coli}$=557.124, $F_{S.\ aureus}$=87.538 and $F_{mixed\ (E.\ coli\ +\ S.\ aureus)}$ = 596.119 all at p<.001). Both bacterial strains in pure culture showed significantly higher biofilm formation (OD mean ± SE) at 30°C (*E. coli* 1.32 ± 0.015 and *S. aureus* 1.26 ± 0.048), compared with 37°C (*E. Coli* 1.14 ± 0.009 and *S. aureus* 0.9 ± 0.024) and 22°C (*E. coli* 0.73 ± 0.012 and *S. aureus* 0.53 ± 0.039) – comparison by *Tukey HSD*, with $p < 0.001$. However, no significant differences were recorded in mixed-species biofilm formation at 30°C and 37°C (1.165 ± 0.013 vs. 1.163 ± 0.014 – *Tukey HSD*, with $p > 0.05$). The results of the trial support the hypothesis of stronger biofilm formation at 30°C in a controlled environment incubation unit.

Keywords: biofilm formation, optimal temperature, CV microplate assay.

Biofilms have been described in many systems ever since Van Leeuwenhoek examined the "animalcules" in the plaque sampled from his own teeth in the 17th century, but the general theory of biofilm predominance was not promulgated until 1978 (4).

It is now known that microorganisms universally attach to surfaces and produce extracellular polysaccharides resulting in the formation of biofilms, considered by many to represent the natural growth state of bacteria (5, 10). This state confers on the bacterial cells a high level of resistance towards conventional antibiotics and disinfectants compared with the planktonic form (6). For this reason,

biofilms cause significant problems in both medical (e.g. device-related infections) and non-medical settings (e.g. food processing environments, drinking water distribution systems) (7, 8, 11).

Unlike the standard minimal inhibitory concentration protocol to assess antibacterial activity against planktonic cells, there is no standardized method to evaluate biofilm formation by clinical bacterial isolates or biofilm inhibition and/or eradication capacity of novel anti-biofilm compounds (10).

The crystal violet (CV) based microtiter plate assay is an indirect method of biofilm biomass quantification in the entire well (3, 19). CV is a basic dye, which binds to negatively charged surface molecules and polysaccharides in the extracellular matrix, as well as to both living and dead cells (12, 14).

Although some researchers managed to improve the CV based microplate assay and to optimize the conditions required for the biofilm formation (19), this simple and reproducible method still exhibits issues with ”the edge effect”, which hampers the usage of the entire microplate, and with the lack of standardization (10, 17, 18). Moreover, when dealing with microorganisms that do not require special growth temperatures, the commonly applied temperature is 37°C. This is due to the relevance of this temperature in infectious diseases. However, in the food production environments and in hospital environments, temperatures both below and above 37°C are applied (15).

Therefore, in the present study we aimed to compare the biofilm formation of two multidrug resistant *E. coli* and *S. aureus* strains, at 22, 30 and 37°C, for 48 h, in a controlled environment incubation unit (invention currently under review for patent) – a system designed to prevent the higher gas exchange and evaporation from the outer wells of the microplate.

Materials and methods

Multidrug resistant *S. aureus* CO0587 (isolated from wound secretion; methicillin resistance phenotype) and *E. coli* CP7921 (isolated from urine sample; fluoroquinolone and sulfonamide resistance phenotypes, penicillinase production) were provided by OncoGen Research Center – “Pius Brinzeu” Timisoara County Emergency Clinical Hospital.

In the present study, the effect of different incubation temperatures on *in vitro* biofilm synthesis was assessed using 96-well clear flat bottom Corning plates.

The isolates were grown overnight on Columbia agar at 37°C. Colonies from overnight cultures were suspended in physiological saline (0.9% NaCl) and mixed to obtain a bacterial suspension of 0.5 McFarland turbidity (1.5 x 10^8 CFU/ml). Each well was filled with 140 µl of sterile BHI broth and 10 µl of bacterial suspension was added. Wells corresponding to the negative controls were filled with 150 µl of sterile BHI broth.

Plates were placed in three different controlled-environment incubation units, which were situated in three classic incubators set at 22, 30 and 37°C for 48

h. The mean temperatures measured inside the incubation units during the 48 h were 22.34 ± 0.12, 30.11 ± 0.42 and 36.97 ± 0.13°C.

After incubation, the plates were inverted and gently tapped to remove residual broth. The wells were washed twice with 200 µl of distilled water to remove planktonic cells. Plates were then incubated for heat fixation at 60°C for 20 min.

Staining was made with 155 µl of 0.1% crystal violet (CV) for 20 min. The staining solution was discarded; plates were gently rinsed twice with distilled water using a wash bottle and tapped on dry paper towels to remove excess water.

The CV bound to the adhered biofilm was solubilized with 160 µl of 96% ethanol and incubated for 30 min at room temperature with gentle shaking. The absorbance was recorded at 540 nm using the Tecan® microplate reader (Fig. 1).

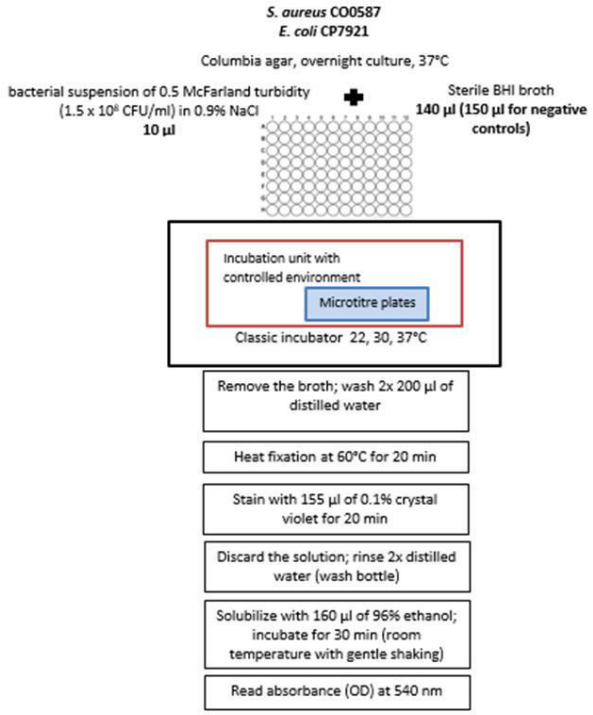

Fig. 1. Schematic representation of the CV based microplate assay

One-way ANOVA followed by Tukey HSD were used to compute and analyse the differences in OD values obtained with different incubation temperatures for *in vitro* biofilm synthesis through CV based microplate assay. The level of significance was considered at $p < .05$.

Results and discussions

The temperature proved to be a significant factor in biofilm formation for both pure and mixed culture biofilms (ANOVA, $F_{E.\ coli}$ = 557.124, $F_{S.\ aureus}$ = 87.538 and $F_{mixed\ E.\ coli\ +\ S.\ aureus}$ = 596.119 all at $p < 0.001$). Both bacterial strains in pure culture showed significantly higher biofilm formation (OD mean ± SE) at 30°C (*E. coli* 1.32 ± 0.015 and *S. aureus* 1.26 ± 0.048) in comparison with 37°C (*E. coli* 1.14 ± 0.009 and *S. aureus* 0.9 ± 0.024) and 22°C (*E. coli* 0.73 ± 0.012 and *S. aureus* 0.53 ± 0.039) – comparison by *Tukey HSD*, with $p < .001$. However, no significant differences were recorded in mixed-species biofilm formation at 30°C and 37°C (1.165 ± 0.013 vs. 1.163 ± 0.014 – *Tukey HSD*, with $p > .05$) (Fig. 2).

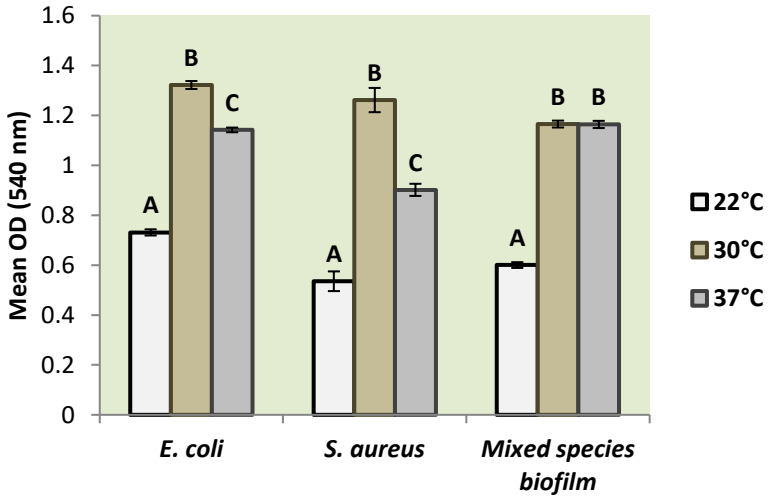

Fig. 2. Biofilm formation at different temperatures
Error bars: +/- 1 SE
In the same category, means with different leters are significantly different (Tukey's HSD, p < .001)

It is well known that the environmental conditions impacts biofilm formation and developement (1). The controlled-environment incubation unit was designed to reduce the „edge effect", by preventing the higher gas exchange and evaporation from the outer wells of the microplate and thus to increase the homogeneity of the results. In order to minimise the excessive water loss and to maintain humidity during the incubation period, some groups of researchers obtained good results by adding physiological saline in the peripheral wells (9, 18). However, this improvement has the major disadvantage of making unusable many of the microtiter plate wells, which otherwise could have been filled with bacterial inoculum. Thus, the method requires

a larger number of microtiter plates which has a negative impact on the time budget and costs of the protocol. By using the controlled-environment incubation unit, the microplates were entirely capitalized and we obtained homogenous results and accurate representations of the true population mean.

When evaluating *in vitro* biofilm formation of clinical isolates or biofilm inhibition and/or eradication capacity of a novel compound, it is important to assure the optimal growth conditions for the bacterial strain of interest. Abdallah et al. (1) reviewed the influence of factors, such as temperature and humidity changes, nutrient availability, oxygen level, pH and surface type, on the biofilm formation of pathogenic bacteria and concluded that the effect of the temperature changes on the biofilm formation of *S. aureus* remains unclear because of the discrepancies between studies undertaken in this regard.

The temperature of 37°C, considered almost the gold standard temperature when it comes to experiments involving biofilm formation, has relevance just in infectious diseases, while in food industry and in hospital environments, temperatures both below and above 37°C can be more applicable (15).

Rode et al. (15) when working with ten different bacterial strains grown in various nutritional matrices found that suboptimal temperatures (46°C, but mostly 25 and 30°C) increase the biofilm formation of *S. aureus*. Instead, Choi et al. (2) and Vazquez-Sanchez et al. (20) found that the biomasses of *S. aureus* biofilms grown at 37°C were more important than those grown at 25°C. However, Pagedar et al. (13) reported a higher cell count of *S. aureus* biofilms at 25°C than at 37°C.

Silhan et al. (16) showed that the survival of *E. coli* in drinking water pipes is very temperature dependent. Temperatures around 15°C were more favourable than 35°C values, even though this is the typical temperature in their natural environment.

Our findings regarding the optimal incubation temperature to be used when assessing biofilm formation successfully complements other studies, in an attempt to standardize a method for quantifying bacterial biofilm.

Conclusions

The results of this trial support the hypothesis of stronger biofilm formation at 30°C, when compared with 22°C and 37°C, in an incubation unit assuring controlled environment.

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CLINICAL LOCALIZATION OF NEUROLOGIC LESIONS AND IMAGISTIC CONFIRMATION IN INTERVERTEBRAL DISC DISEASE IN DOGS

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Summary

Intervertebral disc disease (IVDD) in small breed dogs is a commonly encountered neurological disease and patients may present with various forms of primary complaints, histories and symptoms. Thereby, a complete neurological exam must be performed so as to refer for further imagistic investigation. The aim of this study was to establish the accuracy of neurological localization of spine lesions in IVDD which were further compared with the imagistic examination for confirmation of clinical localization. The clinical aspects were evaluated in 8 dogs between 3 and 12 years old registered between February 2021- May 2021 at the department of Internal Medicine, Faculty of Veterinary Medicine, Cluj-Napoca. The canine patients presented with characteristic symptoms that suggested medullary compression determined by Hansen II type disk hernias. In a constructive research, a neurologic examination was performed on each patient using specific known tools and methods and the NeuroMap System for localization guiding. The dogs with suspected of spinal lesions were registered and staged suitable for each individual. Subsequently a Computer Tomography was performed under general anesthesia. Lesions identified on the CT scans were later compared to the clinical localization. Results of the following study concluded an accuracy of 75% of the clinical localization of spinal lesions in intervertebral disk disease in dogs, numerical aspects considering two of the evaluated patients whom clinical diagnosis was not confirmed by imagistic examination.

Keywords: intervertebral disk disease, neurologic examination, lesion localization, computer tomography, NeuroMap System.

The degenerative process of intervertebral discs is most commonly associated with aging and starts with dehydration processes and loss of proteoglycans in the nucleus pulposus. At the same time the quantity of collagen will increase progressively and the cellularity in the structure will be diminished. Thereby, the modifications in the structure and metabolic processes of the nucleus pulposus and the progressive degeneration of the anulus fibrosus will later be identified with concentric crazings of the anulus fiborsus. These changes can lead to partial or total herniation of the nucleus pulposus in a dorsal plan which will cause medullary compression and significant clinical symptomatology. Chondrodystrophic dog breed such a Daschunds will present additionally to the degenerative common processes of the spine a condroid metaplasia of the

nucleus pulposus, followed by its calcification. This processes are observed even in young individuals representing the chondrodysptrophic breeds in the first two years of life (3, 5, 15, 17, 13).

IVDD is a progressive, chronic affection and disc hernias will materialize as a secondary affection, causing focal inflammation of the intervertebral disc twards the spinal cord. The clinical findings have to be closely observed for an accurate neurolocalization. Considering the affected either upper motor or lower motor neurons, the symptom are specific for affected functional regions of the spinal cord (3, 5, 6, 8).

The aim of this study was to evaluate the accuracy of neurological localization of spine lesions in IVDD which were further compared with the imagistic examination for confirmation of clinical localization.

Materials and methods

The clinical aspects were evaluated in 8 dogs, aged between 3 and 12 years old registered between February 2021- May 2021 at the Department of Internal Medicine, Faculty of Veterinary Medicine, Cluj-Napoca. A complete neurological exam was performed and charted in a neurological report concluding with neurolocalization.

The clinical neurological exam had the following objectives: demonstrating the lesions's appurtanence to the nervous system, evaluating the severity and prognosis of the localized lesions (4, 5, 18, 17). First, a complete history of the patient was obtained, immediately followed by a brief physical examination and evaluating the patient's vitals. Laboratory analysis such as complete blood count and sanguine biochemistry were performed in all patients.

Regarding the neurological exam, there are specific evaluating methods (2, 3, 4, 8, 17, 19, 20) that guide the physician for an accurate neurolocalization, hereby examining each component of the nervous system. The neurological exam instruments consisted of a reflex hammer, pinwheels, a pair of haemostatic forceps, penlight, the neurologic examination form. Initial evaluating is a hands-off examination and concentrated on observing the patient's mental status, behavior and posture. Postural reflexes and proprioception were further evaluated with different techniques such as hemistand, hemiwalk and hopping tests. Examination of the cranial nerves followed and spinal reflexes were subsequently evaluated. If sensibility and superficial pain deficits were observed a deep pain evaluation was further considered. Also, fecal or urinary incontinence were noted. All observations were noted and charted (Table 1).

Table 1

Clinical observations charted in neurological exam for presented cases

Case	Mentation	Gait and Posture	Proprioception	Cranial Nerves	Spinal Reflexes	Deep pain	Incontinence
1	Normal	Lumbar lordosis	Hind limbs deficit	Normal	Normal	Normal	Not presented
2	Normal	Lumbar khyphosis Paraparesis	Severe deficits on hind limbs	Normal	Intensified	Late	Not presented
3	Normal	Thoraco-lumbar khyphosis	Mild deficit on hind limbs	Normal	Clonic muscle contracture	Normal	Not presented
4	Normal	Thoraco-lumbar khyphosis Paraparesis	Mild deficit on hind limbs	Normal	Intensified	Normal	Not presented
5	Normal	Paraplegic	Severe deficit on hind limbs	Normal	Deficit	Absent	Present
6	Normal	Lumbar lordosis	Mild deficit on hind limbs	Normal	Normal	Normal	Not presented
7	Normal	Paraplegic	Severe deficit on hind limbs	Normal	Deficit	Late	Present
8	Normal	Paraparesis	Mild deficit on hind limbs	Normal	Normal	Normal	Not presented

Thereby, obtained data was analyzed and oriented for neurolocalization using the NeuroMap System (4, 8, 10, 17). This system considers and evaluates either upper motor neurons or lower motor neurons dysfunction signs and concludes in Central Nervous System functional regions localization. Patients were later directed to the Department of Radiology and Imagistics, Faculty of Veterinary Medicine, Cluj-Napoca for native Computer Tomography (CT) and contrast CT scanning. Obtained CT scans were archived on a PACS unit and processed by the 3DMedicaland HOROS software for further evaluating and reading of the DICOM files. Clinical findings and neurolocalization were further compared with imagistics findings for evaluating the NeuroMap System's efficiency. Lesions observed on the CT scans can be observed, measured and accurately localized (Fig. 1, Fig. 2).

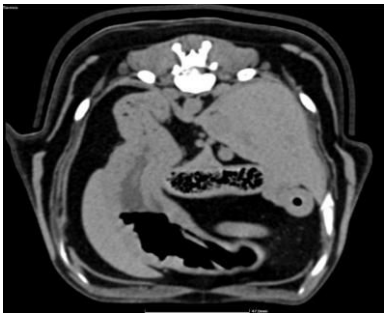

Fig. 1. Disc extrusion T13-L1

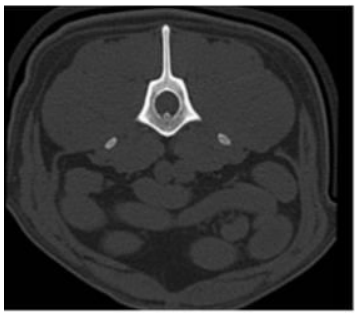

Fig. 2. Disc extrusion L3-L4

Results and discussions

Neurolocalization using the NeuroMap System concluded a most common localization of spinal lesions in the thoraco-lumbar spinal region, more specific in the T3-L3 region (Fig. 3) with a incidence rate of 62.5% of the considered cases. Although clinical neurlocalization techniques are farely accurate and guide the final diagnosis, there are some exceptions of the NeuroMap Systems that were observed in the evaluated patients. Thus, spinal lesion localization in 75% of the total examined cases were confirmed by the Computer Tomograph Scan (Fig. 4). A total of 25% were considered exceptions, due to the fact that clinical findings were not confirmed by the Imagistic investigations. Spinal shock effect was considered a potential cause for clinical presentation exceptions.

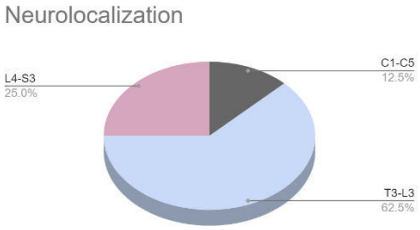

Fig. 3. Neurolocalization of spinal lesions using the NeuroMap

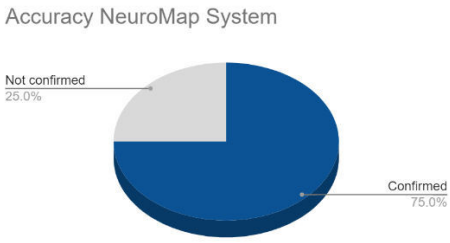

Fig. 4. Imagistic confirmation rate of clinical neruolocalization

Such an example was represented by a 5 years old Daschund, female, who's neurolocalization was evaluated as a lumbo-sacral region (L3-S4) due to the presented symptoms such as diminished reflexes on the hind legs, muscle atrophy, decrease muscle tone, urinary incontinence, fecal incontinence, paraplegic posture

and an overall flaccid paralysis of the hind legs. However, imagistic diagnosis concluded a T12-T13 localization of an discal extrusion (Fig. 5) which would normally determine spastic paralysis, increased muscle tone and a spastic bladder, symptoms that were not preset in the evaluated case.

Clinical staging of IVDD in accordance to BSAVA Manual of Canine and Feline Neurology in considered agreed that most common charted staages were III and IV which included 50% of total patients. 25% of considered cases classified in Stages I and II and 5% in stage V. Clinical staging guided for the severity of spinal cord compression and herniated discs lateralization. In a study conducted by Silke Hecht et.al. in 2009 two different imagistic protocols were evaluated in determining the severity and lateraliations of spinal thoraco-lumbar disc hernias in correlation with surgical and clinical findings. Different types of CT scanning protocols were evaluated in 19 chondrodystrophic dogs. Level of lateralization and localization of disc extrusions with conventional CT had an agreement with surgical findings up to 78.9% an concluded that both conventional and helical CT were accurate in evaluation of acute intervetebral disc hernias.

Franck Forterre et.al. determined in a 2007 study regarding imagistic confirmation of clinical neurological localization of spinal lesions in 23 dogs a accuracey of 70%.

Regarding the incidence of spinal lesions localization, Abdelhakiem et. al. concluded in a study considering 26 dogs presented also a common site of thoracolumbar disc hernias which were detected in 38% of totl considered cases. In the same study, an accuracy of relfexes eamination for neurocaliztion compared with subsequently CT scanning concluded a 71.4% agreement.

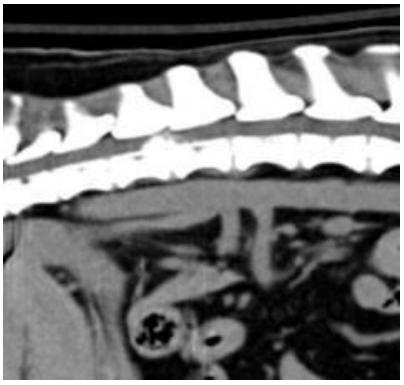

Fig. 5. T13-L1 disc extrusion in a paraplegic Daschund

Conclusions

A complete neurological clinical exam is an essential milestone in accurately localizing spinal lesions and guiding for a final diagnosis and further recommendations. Clinical neurolocalization confirmation using special imagistics methods is an important and necessary phase due to the clinical cases that don't fit into classical clinical presentation.

Clinical standardized Staging of IVDD can guide for severity and prognosis ealuation.

The accuracy of clinical neurolocalization was confirmed by CT scans for a total of 75% in the evaluated cases.

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CAMELPOX, CURRENT STATUS, EPIDEMIOLOGY AND CHALLENGES

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Summary

Camelpox is considered an emerging public health problem due to increased reported cases and outbreaks in camels during this decade. Camelpox is a highly contagious skin disease of camelids caused by camelpox virus (CMLV), a member of the genus *Orthopoxvirus* within the family *Poxviridae*. The disease is often manifested as a mild local skin infection and sometimes in severe form with systemic involvement. Camelpox virus is very host specific and does not infect other animals. The disease is enzootic in the camel-rearing areas of arid and semiarid regions of the world and causes economic loss in terms of morbidity, mortality, loss of weight, and reduction in milk and wool production. The CMLV infection is transmitted mostly by direct contact and aerosol route. Zoonotic camelpox virus infection in humans associated with outbreaks in dromedary camels (Camelus dromedarius) was described in the north-eastern region of India during 2009. This was a single incident illustrating that camelpox is of limited public health importance. A very strict surveillance for any camelpox outbreak with particular attention for any human case has to be implemented. This work is timely because very strict surveillance must be implemented for any outbreak of smallpox with special attention to any human case.

Keywords: camelpox, epidemiology, Orthopoxvirus.

Camelpox virus infection causes a severe generalized disease in camels and dromedaries that is characterized by extensive skin lesions. It is an important disease, especially in countries of Africa, the Middle East, and southwestern Asia, where the camel is used as a beast of burden and for milk (1, 5, 7, 18, 19). The more severe cases usually occur in young animals, and in epizootics, the case-fatality rate may be as high as 25%. The causative virus is a distinctive orthopoxvirus species, and comparative genome analysis shows camelpox virus to be closely related to other orthopoxviruses, including the variola virus (smallpox virus). Genomic differences that distinguish camelpox from other orthopoxviruses occur in genes that probably determine either host range or virulence (9, 10, 11, 17). Camelpox virus has a narrow host range, and despite the frequent exposure of unvaccinated humans to florid cases of camelpox, human infection has not been described. A parapoxvirus (Ausdyk virus) also infects camels, producing a disease that can be confused with camelpox (2, 5, 8, 15, 16).

The disease is one of the most important infectious diseases of camels in Ethiopia, Somalia. In Ethiopia, a clinical prevalence of between 0.45% and 14.2% has been reported in different parts of the country (12, 15, 18).

Materials and methods

The epidemiological data were obtained from the site-ul Office International des Epizooties - World Animal Health Information System (OIE –WAHIS). Camelpox is considered an emerging public health problem due to increased reported cases and outbreaks in camels during this decade. Zoonotic camelpox virus infection in humans associated with outbreaks in dromedary camels (Camelus dromedarius) was described in the north-eastern region of India during 2009 (4, 8, 11). Very strict surveillance for any camelpox outbreak with particular attention for any human case has to be implemented. This work is timely because very strict surveillance must be implemented for any outbreak of smallpox with special attention to any human case.

Results and discussions

Camelpox is a common cause of camel morbidity under traditional nomadic management systems in developing countries but this disease is less significant in developed countries, where it incurs less threat to man or animals. The threat CMLV poses to people whose well-being depends on the health of their camels makes the disease of considerable economic and public health importance. CMLV is a zoonotic agent and mostly host-specific but recently evidence has been documented from Somalia in smallpox-unvaccinated individuals and from India in smallpox-unvaccinated camel handlers or attendants. Mild skin lesions in humans associated with camelpox have been reported, indicating camelpox may be of public health impact. Among the human cases, people drinking milk from camelpox-affected animals have been reported to develop ulcers on the lips and in the mouth, but these observations could not be visualized or laboratory-confirmed (2, 5, 10). However, under certain conditions, the virus could be pathogenic for human-like that like cowpox and monkeypox, especially in immune-compromised individuals (13, 14, 15). However, no systematic epidemiological studies have been undertaken on human cases due to the lack of immunological surveys for specific camelpox antibodies among unvaccinated herds (4, 7, 10, 13, 17).

In Asia, in 2014 January-June most outbreaks of the disease were reported (11), followed by 2014- 9 outbreaks, 2019- 8 outbreaks, and 5 outbreaks in 2010, 2012, 4 outbreaks in 2011. In 2011 and 2016 the disease was not reported to be present in Asia (Fig. 1).

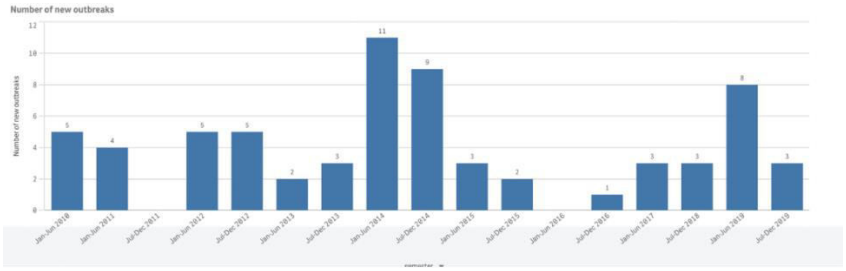

Fig. 1. The camelpox outbreaks in Asia (20)

In Africa, the situation is totally different so analyzing the data in the graph we can see that in 2013 most outbreaks of the disease were reported (11), and in 2010 the first semester, 2015, and 2016 the disease was not reported (Fig. 2).

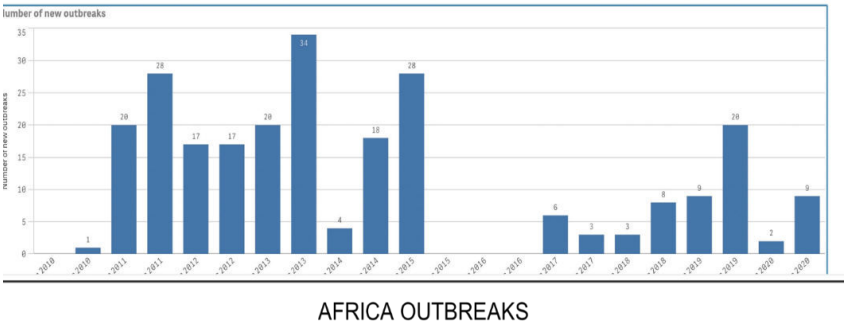

Fig. 2. The camelpox outbreaks in Africa (20)

Conclusions

Camelpox is a ***socio-economic concern*** as it incurs a considerable loss in terms of morbidity, mortality, loss of weight, and reduction in milk yield for the animal, in addition to economic losses to the camel racing and transportation industries.

Education of the people about the etiology of camelpox, strict separation of diseased and healthy young camels, improved health care including long-acting antibiotics, improved hygiene, and general supportive treatment will decrease the harmful effects of camelpox.

Further epidemiological studies of camelpox on these endemic regions are necessary to assess the circulation of CMLV, both in camels and humans in order to know its public health significance.

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EPIDEMIOLOGY OF EQUINE PIROPLASMOSIS AND ITS ASSOCIATED RISK FACTORS IN EUROPE: A REVIEW OF THE LAST 21 YEARS

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Summary

Theileria equi and *Babesia caballi* are the primary pathogens of the intraerythrocytic parasitic disease, equine piroplasmosis (EP). Natural transmission of EP occurs via specific ixodid tick vectors, of which, *Ixodes, Haemaphysalis, Dermacentor* and *Rhipicephalus* species have been described. Numerous articles on EP have been published in the last decade. This study aims to characterize the circulation of EP in European countries and associated risk factors, as well as to have an overview of current knowledge. In order to achieve the aim of this study, a systematic database search was carried out, resulting, after a preliminary selection, in a total of 32 publications considered eligible. By combining all relevant studies carried out in Europe on the circulation of piroplasms, we determined that the prevalence of *B. caballi* and *T. equi* in Europe is 10.15% (0% - 47.8%) and 25.36% (1% - 66%), respectively, and the total prevalence is 35.51% (1.5% - 88.4%). The most studied variables in risk factor analyses performed in European countries were individual characteristics, with different results. As some countries have several published studies while others have only one, the estimated prevalence and seroprevalence should be interpreted with caution. This study also identified a number of European countries, including Romania, for which there is insufficient information on the epidemiology of EP. The lack of knowledge of the spread of piroplasms species and their vectors represents a risk both for these countries and for the rest of Europe.

Keywords: *Babesia caballi*, *Theileria equi*, equine piroplasmosis, risk factors, Europe.

Theileria equi and *Babesia caballi* are the main pathogens of the intraerythrocytic parasitic disease, equine piroplasmosis (EP) (47). Natural transmission occurs via specific vectors, ixodid ticks (40), of which, *Ixodes, Haemaphysalis, Dermacentor* and *Rhipicephalus* species have been described (41). A third species capable of infecting equines, *Theileria haneyi*, has recently been discovered in North America (20), but has not been yet reported in Europe.

It is endemic in most of the world, with serious clinical and economic consequences but also due to the negative impact on international trade (26, 22) caused by transport restrictions with non-endemic countries (38, 47, 46). In non-endemic countries, to prevent the introduction of carrier animals, only seronegative horses are allowed to be imported (14).

Only a few countries, such as Japan, USA, Canada, Northern Europe, Iceland, Greenland, New Zealand and Australia, are recognized as non-endemic.

EP is an OIE List B disease, communicable diseases that are considered to be of socio-economic and/or public health importance within countries and are significant in international trade in animals and animal products (50).

Although the majority of EP endemic areas are within tropical and temperate zones, continuous changes in climatic conditions as well as the transport of untested animals have led to the spread of both parasites and vectors into previously non-endemic areas, an example being UK (10).

In the last two decades numerous articles have been published on the accumulated information on the prevalence of T. equi and B. caballi but also on the risk factors associated with these infections (7, 21, 30, 31, 39, 40, 44, 46, 47).

The present study aims to characterize the circulation of EP in European countries and associated risk factors as well as to have an overview of current knowledge.

Materials and methods

To achieve the aim of this study, a systematic multi-stage search of Pubmed and Science Direct databases was conducted to identify all studies falling within the scope of the topic.

First, the keywords "equine piroplasmosis" were entered together with "Europe" to geographically limit the studies searched (i). Articles between 2000 and 2021 were selected (ii) and had prevalence/seroprevalence and case reports as their design (iii). As *B. equi* was recently (1998) renamed *T. equi* (26), the term "*Theileria equi*" was entered into the databases.

The key terms "equine" and "equine piroplasmosis" have allowed identification of studies in both horses and donkeys.

After selecting papers based on titles and abstracts, studies were further analyzed by detailed examination of the full text (Fig. 1). Articles that were included in the study had to meet all of the following criteria:

(i) original research articles with geographical limitation (Europe);
(ii) research conducted between 2000 and 2021;
(iii) study design to be prevalence/seroprevalence or case reports;
(iv) diagnostic method clearly specified;
(v) the geographical location of the study is clearly stated;
(vi) the number of positive cases and sample size are clearly stated.

The search resulted in 429 articles, which were then checked to establish the purpose of the work, the diagnostic methods used and the animals studied.

Only studies that involved European countries and that related to epidemiological characteristics of EP were selected for analysis. After a preliminary screening of the studies a total of 32 publications were considered eligible.

Results and discussions

One of the main objectives of this review was to study the circulation of both piroplasma species among equines at a more restricted level.

Of the 32 articles, 29 were selected and analyzed in terms of prevalence and seroprevalence of *Theileria equi* and *Babesia caballi* and associated risk factors, and 3 papers presented case reports in domestic or imported horses and transplacental transmission of *Theileria equi*.

By combining all relevant studies conducted in Europe on the circulation of piroplasms, we determined that the seroprevalence of *B. caballi* and *T equi* in equines in Europe is 10.15% and 25.36%, respectively, and the total seroprevalence is 35.51%.

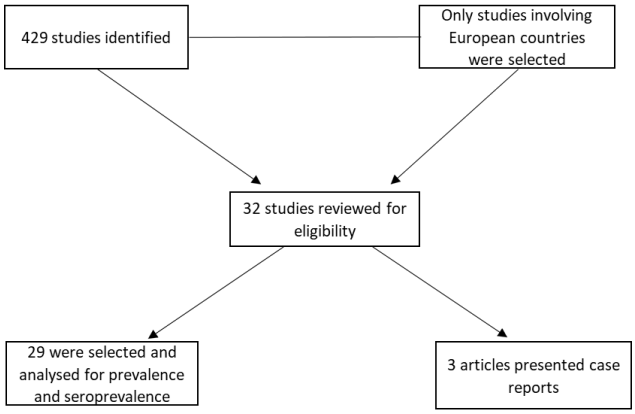

Fig. 1. Study selection process

Table 1

Data on prevalence and seroprevalence in Europe

No	Country	No.	*Theileria equi*	*Babesia caballi*	Mixed infection	Overall Prevalence	Reference
1	Czech Republic	771	1.1%	0.4%	-	1.5%*	(3)
2	Switzerland	689	4.4%	1.5%	1.5%	7.3%	(42)
3	France	443	58 %	12,9%		70.9%*	(18)
4	Greece	7872	12.9%	1,3%	-	14.20%	(25)
5	Greece	544	11%	2,2%	1.7%	11.6%	(21)
6	Ireland	2099	2.5 %	1%	-	3.5 %	(10)

7	Italy	520	9.3%	-	-8	9,3%	(32)
8	Italy	135	13,33%	0%	-	17.04%	(48)
9	Italy	673	39.8%	8,9%	-	48.7%*	(2)
10	Italy	138	40.6%	47.8%	19.6%	88.40%*	(23)
11	Italy	203	44,3%	35,5%	22.6%	57.1%	(35)
12	Italy	300	41%	26.0%	-	67%*	(24)
13	Italy	294	8.2%	0,3%	-	8,5%	(17)
14	Italy	412	12,4%	17,9%	38.1%	68.4%	(28)
15	Netherlands	300	1%	3%	-	4%	(4)
16	Poland	512	7.2%	-	-	7.2%	(45)
17	Portugal	162	17.9 %	11.1%	-	29%	(37)
18	Romania	193	20.3%	2,2%	3%	25.4%	(15)
19	Serbia	70	50%	0%	-	50%	(11)
20	Serbia, Montenegro, Bosnia and Herzegovina	142	22.5%	2,1%	0.7%	25.4%	(12)
21	Spain	740	35.8%	15.6%	8.9%	42.9%	(6)
22	Spain	3368	21%	5.6%	2.5%	24,1%	(7)
23	Spain	235	66.0%	29.4%	-	72.8%	(27)
24	Spain	3100	44%	21%	12.84%	52%	(27)
25	Spain	135	17,03%	2,96%	-	19.99%	(1)
26	Spain	380	56,1%	13,2%	10.8%	58.4%	(16)
27	Spain	60	40%	28,3%	20%	68.3%*	(5)
28	UK	1242	5.9%	4.4%	27.1%	8.0%	(9)
29	Hungary	324	32.0%	-	-	32.0%	(13)

*Total prevalence calculated from data provided in the articles studied.

Between 2000 and 2021, a total of 26 576 animals were studied in Europe, of which 23 329 were horses and 491 donkeys. Of the 29 articles analyzed, 5 were carried out on/with donkeys.

Risk factors for equine piroplasmosis

Of the 32 articles selected for this study, 10 were concerned with risk factors for EP. In almost all studies the authors referred to risk factors such as breed, age, sex, activity, shelter, region, sampling period, and differences in prevalence between the two species.

The most studied variables in risk factor analyses in European countries were individual characteristics, with age and sex being the most frequently analyzed.

Findings on the association between age category and *T. equi* seropositivity showed discrepancies between studies. Most studies found age to be significantly associated with *T. equi* seropositivity (2, 18, 21, 42, 48) with older animals being more commonly affected than younger ones, while other studies found no significant differences between age categories (23, 24, 28, 32, 37). Older age has been considered a risk factor for seropositivity, explained by longer exposure to tick vectors.

Only one study reported higher seropositivity for young, imported animals compared to older, indigenous animals ($p<0.0005$) (32).

Findings on gender as a risk factor had even greater discrepancies between studies, when the difference was significant between males and females it was usually considered to be related to management practices that differed by gender. For example, some studies have identified sex as a protective factor against *T. equi* and/or *B. caballi* infections, with males being less susceptible (18, 28, 39).

Prevalence differences between the two piroplasm species have been analysed in at least 7 articles, most of them concluding that the prevalence for *T. equi* is significantly higher than for *B. caballi*, $p=0.002$, (32, 6), except for one study in which the seroprevalence *for B. caballi* was higher, but without statistically significant differences ($p=0.3$) (23).

The type of activity practiced was also a risk factor analyzed in several studies, for example farm horses had a higher risk of infection than race horses ($p<0.01$), and race horses had a higher risk than recreation horses ($p<0.05$) (21, 48).

Although some authors state that mixed infections are common (4, 6, 7, 23, 27, 35), there are studies in which no positive samples have been reported for both species (9, 11). On the other hand, some authors have identified mixed infections only in indigenous animals, with the difference in seropositivity being significant between imported and indigenous animals, $p<0.05$ (21).

Animal species was found to be a significant risk factor for the presence of *T. equi* and/or *B. caballi* infections, $p<0.0001$ (21).

A significant increase was observed for *B. caballi* in samples collected in autumn and winter ($p =0.030$) which could not be demonstrated for *T. equi* ($p=0.240$). The spread of *B. caballi* to horse populations in summer may be translated by increased antibody titers detected by serological methods in autumn and winter.

It is important to note that different genotypes of *T. equi* (referred to as A-E) circulate in Europe, which may ultimately explain some differences in prevalence between countries, even though no link between genotype and virulence has been established so far (46).

However, as some countries have several published studies while others have only one or none, the estimated prevalence and seroprevalence should be interpreted with caution, thus in Spain and Italy a number of 7 and 8 articles respectively have been published analyzing prevalence, while in countries such as the Czech Republic, Ireland, Poland, the Netherlands, Portugal, Serbia, Hungary but also Romania, only one study has been conducted.

Prevalences for *T. equi* are higher than those observed for *B. caballi*, a difference that can be explained by the fact that *T. equi* infection is lifelong in equines (29, 30, 38, 39, 47), whereas for *B. caballi*, parasite clearance is estimated from 1 to 4 years (29).

In Romania, the first study on EP prevalence was conducted by Gallusová et al. in 2010-2012, which resulted in a prevalence of 25.4% for both piroplasma species by PCR molecular method from 18 localities inside and outside the Danube Delta (15). Also, Ionita et al. (19) reported the first molecularly confirmed study by sequencing *B. caballi* DNA in clinically affected horses.

In Serbia, one of Romania's neighbors, in 2016, a study on 70 donkeys concluded an overall prevalence of 50% for *Theileria equi*, while no sample was positive for *Babesia caballi* (11), while in southern Italy, out of 203 donkey serum samples tested, 72 (35.5%) were positive for *B. caballi* and 90 (44.3%) for *T. equi* (35).

Previous studies in Europe have shown a wide range of seroprevalences for equine piroplasmosis. An overall prevalence of positive samples was 32.0% with ELISA as well as IFAT in a 2013 study of 101 animals on *T. equi* infection in horses in Hungary (13). In Greece, the seroprevalence for *T. equi* and *B. caballi* was found to be 11% (8.6-14%) and 2.2% (1.2-3.9%) respectively (21).

In Switzerland, a total of 50 (7.3%) horses were seropositive for EP, in comparison, within the study seropositivity is reported in native horses (animals bred in Switzerland) and imported horses as 4.8% (11/230) and 8.5% (39/459), respectively (32).

In Germany, EP is considered as an imported parasitic disease. One case reported by Springer et al. (43) in 2019, was of equines that developed clinical infection with *T. equi* after a trip in southern France. A vector tick, *Rhipicephalus bursa*, was identified on one of the animals, a species that the authors claim cannot spread in Germany under current climatic conditions.

Both the UK and Ireland were considered free of EP, and positive cases were most likely imported, but parasite movement was closely monitored in these countries.

Thus, Coultous et al. (9) conducted one survey each from 2013-2016 in both Ireland and the UK, resulting in an overall prevalence of 3.5% and 8% respectively.

Of the total number of samples tested in the UK, 81.5% were samples tested for export and only 0.1% for import. However, the number of currently seropositive horses that have been imported into the UK in the past is not known. Two cases of transplacental transmission of *T. equi* have also been reported in two UK born and bred steers (34).

According to the results of the literature review, Spain, France and Italy seem to be the most affected countries in Europe with a prevalence exceeding 50%.

In asymptomatic horses, parasites can be sequestered in blood vessels, spleen, bone marrow (47), which may explain negative PCR results in an animal with a positive serological result.

In European countries with only a few cases of EP so far, OIE recommendations should be followed to avoid the spread of *B. caballi* and *T. equi* in the near future. The OIE recommends that veterinary authorities in importing countries require the presentation of an international veterinary certificate attesting that the animals subject to export showed no clinical signs of piroplasmosis, that they were subjected to diagnostic tests for equine piroplasmosis 30 days prior to shipment with a negative result but also that preventive vector treatments were carried out within the last 30 days (50).

Conclusions

In conclusion, this study has identified a number of European countries, including Romania, for which there is insufficient information on the epidemiology of EP but also countries where this information is lacking, which indicates a lack of knowledge on the circulation of piroplasm species and their vectors and represents a risk both for the countries concerned and for the rest of Europe.

More studies are needed to investigate as many countries as possible to allow better control using molecular methods, including tick transmission dynamics and geographical distribution of vector species.

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RELATIONSHIP BETWEEN THE EVOLUTION OF THE PUERPERIUM MILK KETONE BODIES VALUES AND THE REPRODUCTION PARAMETERS IN DAIRY CATTLE

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Summary

Data generated by 176 cows in the days 4-80 postpartum were collected between March 2019 - July 2020, animals come from four different farms by way of maintenance, feeding and breed. According to the clinical data, the cows were divided into two groups: group 1 – cows with pathology of the reproductive system, and, group 2- cows without pathology of the reproductive system. BHB (beta-hydroxybutyrate), average number of inseminations for a new gestation, service period and milk protein / fat ratio were statistically reported at season and breed for the both groups. According to the data available in the literature, the animals studied were classified in terms of the clinical evolution of ketosis, comparing the concentration of beta-hydroxybutyrate the results were as follows: for the group 2: 86.2% were negative, 3.44 % suspicious and 10.34% positive; in the group 1: 55.68% were negative, 9.10% suspicious and 35.22% positive. The average number of inseminations needed for a new gestation was 1.9 for the group 1 and 1.36 for group 2. The mean of the variables BHB, service period, protein / fat ratio and the number of inseminations nedeed for 1 gestation indicates influences of ketosis on the cow's puerperium.

Keywords: ketosis, reproduction, beta-hydroxybutyrate.

The onset of lactation is a significant period for the metabolism of cows, because the energy intake is lower than necessary for production, so there is a negative energy imbalance that can lead to a susceptibility to the development of diseases such as infectious and metabolic diseases (3). Metabolic imbalance is part of production diseases, diseases associated with malnutrition or inadequate administration of feed with a considerable frequency in early lactating cows (3, 21) but cows unable to adapt to this transition develop hyperketonemia, a disorder defined by elevated concentrations of ketone bodies such as beta-hydroxybutyrate (BHB), acetone and acetoacetate in the blood (14). Santschi et al. (23) reports an incidence between 25 and 60%. The presence of this pathology leads to economic losses through low milk production, high treatment costs (20) and the loss of the animal by slaughter (23). A. Benedet et al. (2) note that few studies have used BHB concentration in milk to detect hyperketonemia, so given the limitations of tests and standardization of threshold values, there is no unanimously accepted threshold for the clinical picture. BHB concentration values in milk for grading cows with

hyperketonemia (HYK) have recently been proposed as being classified as follows: <0.15 mmol / l - negative, 0.15-0.19 mmol / l suspect; ± 0.20 mmol / l-positive. Koeck et al. (17) and Santschi et al. (23). On the other hand, Lee et al (2016) considered an impairment of HYK cows as between 0.01 and 0.20 mmol / l and affected by clinical ketosis with a concentration equal to or greater than 0.20 mmol /it.

Geishauser et al. (10) compared several tests in cow's milk to detect subclinical ketosis and found a sensitivity between 5 and 75% and specificity between 89 and 100% (excluding a test with a specificity of 55%). Similar values were reported by Geishauser et al. (10) Carrier et al. (4) by comparing milk tests with urine tests.

In order to bring greater stability to the screening of ketosis-prone cows, the chemical methods for detecting ketone bodies have been correlated with FTIR (Fourier transform infrared) spectrometry methods, so the author mentions a correlation around 0.80. Using thresholds of 0.15 mmol / l for Ac and 0.10 mmol / l for BHB, high values for Ac or BHB were detected with a sensitivity of 69 to 70%, a specificity of 95%, with 25 to 27% false positive and 6 to 7% false negative (22).

Among the tests used to diagnose ketosis in cows was the determination of milk fat protein ratio, Duffield et al. observed that the best threshold for diagnosing subclinical ketosis in the first 65 days of lactation was greater than 1.33, but the sensitivity and specificity were only 58% and 69%, respectively. Heuer et al observed that cows with a threshold higher than 1.5 increase their risk of ketosis, displaced abomasum, ovarian cysts, body weight loss and mastitis. Toni et al. note that cows with a protein-fat ratio of more than 2.0 to 7 days postpartum have a higher incidence of placental retention, abomasum displacement, metritis and subclinical endometritis, are more risk of being slaughtered.

Other data, such as dry matter intake (13), as well as some clinical tests, can be used to identify cows at risk of developing ketosis, but they require special devices, labor, long time and therefore are not easy to implement on farms. In addition, these data will mainly detect clinical cases and may omit subclinical cows. A cheap, safe and fast alternative to screening cows at risk of developing ketosis is to detect the concentration of BHB in milk (6).

The aim of this paper was to describe the involvement of hyperketonemia in the apparition of reproductive diseases in the puerperal period and the involvement of hyperketonemia in the installation of a new pregnancy.

Materials and methods

The study was conducted between March 2019 and July 2020, on a total herd of 175 dairy cows on days 4-80 of the puerperal period, the animals were classified according to the farm of origin (Table 1), season (Table 2) and breed (Table 3).

The animals come from four farms according to Table 1, three located in Satu Mare county and onelocated in Bistrița Năsăud County, in a proportion close to

the maximum limit, they have only one breed on the farm, so in order to have a homogeneity in terms of breed, cows whose genetic basis is not predominant on the farm were excluded from the study. In this way, three breeds with provenance and frequency presented in table number 3 were registered in the study.

The laboratory data were provided by the Foundation for milk quality control Cluj, through the program, Associations for the genetic improvement of cattle. After harvesting the milk samples were analyzed using the Foss Milkoscan, this is a fully automatic analyzer with a high test capacity (up to 600 samples per hour). It uses the Fourier transformer (FTIR), with infrared, it measures a complete range of milk parameters such as: fat, protein, lactose, urea, citric acid, fatty acids, fatty acid profile, pH, ketone bodies, being according to ISO / IDF and AOAC.

Detection of ketone bodies in milk with the device Milkoscan FT + has a sensitivity between 69 and 87% and a specificity of 95% (22).

Table 1

Distribution of animals according to origin

Firm name		Number of animals / farm	Percent	Validated percentage	Cumulative percentage
validity	Farm (A)	47	26.9	26.9	26.9
	Farm (B)	70	40	40	66.9
	Farm (C)	24	13.7	13.7	80.6
	Farm (D)	34	19.4	19.4	100
	Total	175	100	100	

Table 2

Distribution of animals according to season

		Number of animals	Percent	Validated percentage	Cumulative percentage
validity	Spring	52	29.7	29.7	29.7
	Summer	34	19.4	19.4	49.1
	The autumn	33	18.9	18.9	68
	Winter	56	32	32	100
	Total	175	100	100	

Table 3

Distribution of animals according to breed

		Number of animals	Percent	Validated percentage	Cumulative percentage
validity	German pond (BG) Farm B	70	40	40	40
	Romanian Baltata (BR) Farm A and C.	71	40.6	40.6	80.6
	Red Holstein (RH) Ferma D	34	19.4	19.4	100
	Total	175	100	100	

The collection of data on reproductive status was possible with the support of farmers and veterinarians on farms, more specifically data on the evolution of the puerperium were obtained through frequent visits to farms and close collaborations with the veterinarians responsible for the farm, subsequently the acquired data were confronted with the values presented in the analysis bulletins. In the evolution of the postpartum period, a number of 88 cows were diagnosed, which developed pathologies such as: placental retention, uterine prolapse, metritis, endometritis, salpingitis and hormonal dysfunctions, these being introduced in the control group. A number of 87 cows with a physiological evolution of the puerperium, the parturition being in the same time interval with the control group, were introduced in the control group. The service period was determined from the breeding and calving registers, so for each cow it was established with certainty only at the moment of confirmation of gestation,

The data were analyzed withusing the IBM SPSS Statistics 22 application as a research method, Anova. Dependent variables such as physiological or pathological evolution of the puerperium, race, servive period, season, number of inseminations and independent variables such as BHB concentration and protein-fat ratio were taken into account.

Results and discussions

Betahydroxybutyrate concentrations were analyzed from a seasonal perspective, so the highest average value for the group 1 was recorded in spring 0.56 mM / l (n = 26), in summer an average of 0.1114 mM / l was recorded (n = 17), the average autumn was 0.2524 mM / l (n = 17) and the lowest average value was recorded in winter 0.0904 mM / l (n = 28) ($p < 0.05$). Compared to the group 2, the average trend of values was correlated with the group 1, so the highest values were in the spring months 0.1592 mM / l (n = 26), summer 0.0512 mM / l (n = 17), autumn 0.056 mM / l (n = 16), in winter being an average value of 0.0311 mM / l (n = 28) ($p < 0.05$) From the perspective of the relationship of the season with the service period

and the protein fat ratio for both groups (control and control) no correlation and significant differences were established (p> 0. 05). Thus, for the service period there was an average of all seasons of 136.12 (n = 88) (Table 4) with an interval between 116.32-150.58 corresponding to the group 1 and an average of 85.2 (n = 87) (Table 5) for group 2 with limits between 88.32-87.41. The protein fat ratio was between 0.72-0.86 with an average of 0.81 (n = 88) for the group 1 and with limits between 0.30-0.48 and an average of 0.38 for group 2 (n = 87).

Table 4

BHB ratio, service period group 1

Race		Service period	BHB mM / l	Report
BG	Mediate	140.9714	. 2920	. 7820
	N	35	35	35
	Std. Deviation	51.64157	. 30926	. 65610
BR	Mediate	129.8333	. 2661	. 7897
	N	36	36	36
	Std. Deviation	51.69277	. 40632	. 43751
RH	Mediate	139.4706	. 2124	. 9335
	N	17	17	17
	Std. Deviation	35.77555	. 46869	1.04686
Total	Mediate	1.361.250	. 2660	. 8144
	N	88	88	88
	Std. Deviation	48.79156	. 38083	. 67099

Table 5

BHB ratio, service period group 1

Race		Service period	BHB mM / l	Report
BG	Mediate	94.1714	. 1111	. 4137
	N	35	35	35
	Std. Deviation	25.01351	. 12445	. 34432
BR	Mediate	79.2226	. 0680	. 3877
	N	35	35	35
	Std. Deviation	2,047,680	. 13534	. 30741
RH	Mediate	79.0588	. 0300	. 3429
	N	17	17	17
	Std. Deviation	15.18005	. 03518	. 25826
Total	Mediate	85.2069	. 0779	. 3894
	N	87	87	87
	Std. Deviation	22.59921	. 12057	. 31195

From the point of view of the ratio between BHB, service period, fat-protein ratio with the breed, no significance was established, (Table 4, 5) so regarding the evolution of ketosis with involvement in the puerperal period in cows could not be established in this regard that certain individuals with common genetic traits may be more affected.

The percentage of animals affected by hyperketonemia was estabilished according to the parh values of BHB in milk proposed by Koeck et al. (17) and Santaschi et al. (23). For the group 1, the value range of BHB was between 0.00 and 2.00 mmol / l, the percentage being as follows: negative-55.68%; suspect-9.10%; positive-35.22%. Compared to the group 2, the value range was between 0.00 and 0.59 mmol / l and the percentage was as follows: negative 86.20%; suspect 3.44%; positive 10.34%. The prevalence for all animals studied was 29.14% (n = 175) in the suspicious and positive categories (BHB <0.15mmol / l), similar to Santachi et al. (23) reports in a study of milk obtained from the Holstein breed, in the days 5-35 postpartum a percentage of 22.9% for the same categories (suspects, positive). Compared to the prevalence determined by measuring BHB in the blood, the situation is as follows, Suthar et al. (25) obtained an overall prevalence of hyperketonemia of 21.8% in 10 European countries. Garro et al. (8) tested postpartum cows between days 4 and 19, which were maintained on pasture and reported a prevalence of hyperketonemia of 10.3%, similar Mahrt et al. (19), observed a prevalence between 9.6 and 14.6%.

The period from parturition to the installation of a new gestation has as optimal values between 60-90 days postpartum, exceeding this period leads to economic losses of the farm by decreasing milk production, costs generated by semen, decrease in the number of products for life productive of the animal. Economic losses due to non-optimal fertility are expressed in costs per day of maintenance, Chaidate et al. (5) determined the costs between 0.57-0.70 euro / cow / day. Taking into account the losses recorded, associations were made between the level of ketone bodies and the reproductive performance of cows for the next gestation (12, 24). Getachew (11) mentions that cows diagnosed with ketosis (BHBA≥1.2 mmol / L) in the first week after calving decreases the chances of a new gestation at the first insemination. Compared to the information available in the literature, we identified for each animal included in the study the number of inseminations for the installation of a new gestation, so the statistical analysis reports significant differences between the two groups (Table 8). Of the total number of animals included in the study, a percentage of 6.9% was eliminated, due to the lack of artificial insemination until at least 200 days postpartum.

The results obtained by Andrew et al. (1) on a herd of Holstein-Friesian cows indicate an average of 2.8 ± 0.3 insemination / gestation for the group of cows diagnosed with ketosis and cows there were the ketosis was not present the average was 2.0 ± 0.1 and the success rate for first insemination in ketosis cows indicates an index 4.3 times less likely. Indeed, there are many dependent factors such as breed, milk production, feed, etc., but the trend of comparative values between the two

groups indicates a significance towards the possibility that ketosis can be assimilated with a low conception rate.

Regarding the season, the relationship between the season and the number of inseminations / gestation was established, an average between 1.40-1.78 was obtained, being distributed as follows: spring 1.77; summer 1.78; fall 1.57; winter 1.40 inseminations/gestation. According to statistical interpretations of these results there is no significance between the two variables (p> 0.05).

Determining the ratio between BHB variables and the number of inseminations / gestation in the animals in the study (n = 163) indicates a significance equal to 0.072, so from the point of view of statistical interpretations does not indicate a significant ratio, but the numerical differences are not very far from p = 0.05 which indicates that for these variables are still necessary deeper more accurate results.

Table 6

BHB ratio, service period, fat-protein in each farm, group 1 (p> 0.05)

The farm		Service period	BHB mM / l	Fat / protein ratio
(A)	Mediate	103.5532	, 2283	, 6132
	N	47	47	47
	Std. Deviation	42.39709	. 37495	. 40597
(B)	Mediate	117.5714	. 2016	. 5979
	N	70	70	70
	Std. Deviation	4.666.798	. 25111	. 55221
(C)	Mediate	107.5	. 0513	. 5492
	N	24	24	24
	Std. Deviation	55.28346	. 07595	. 47247
(D)	Mediate	109.2647	. 1212	. 6382
	N	34	34	34
	Std. Deviation	40.89403	. 34010	. 80841
Total	Mediate	110.8114	. 1725	. 6031
	N	175	175	175
	Std. Deviation	45.76682	. 29765	. 56447

Table 7

BHB ratio, service period, fat-protein in each farm, group 2 (p> 0.05)

			Sum of Squares	df	Mean Square	F	Sig.
Service * Farm	Between Groups	(Combined)	6.019.400	3	2.006.467	. 957	. 414
	Within Groups		358.441.378	171	2.096.148		
	Total		364.460.777	174			
BHB * Farm	Between Groups	(Combined)	. 648	3	. 216	2.501	. 061
	Within Groups		14.768	171	. 086		
	Total		15.415	174			
P / g ratio * Farm	Between Groups	(Combined)	. 118	3	. 039	. 122	. 947
	Within Groups		55.322	171	. 324		
	Total		55.441	174			

Table 8

Statistical ratio between the control group and the control group compared to the number of artificial inseminations

			Sum of Squares	df	Mean Square	F	Sig.
Nr. IA / gestation * Lot	Between Groups	(Combined)	11.832	1	11.832	18.939	. 000
	Within Groups		100.585	161	. 625		
	Total		112.417	162			

The interpretation of the clinical results indicates an average service period for the group 1 as being located at 136.12 days (n = 88) compared to the group 2 where the average was 85.2 days (p <0.05). Referring to the value of losses for non-optimal fertility of cows, indicated by Chaidate et al. (5) can reach amounts between 29 and 36 euros / animal. Referring to the number of inoculations / gestation, we mention a difference of 0.54 doses of semen in the group 1, which indicates a lower fertility rate for cows with higher beta-hydroxybutyrate concentrations.

Negative effects on reproductive efficiency due to the incidence of ketosis are confirmed in this study, compared to previous studies confirming long-term effects of ketosis such as, low conception rates at first inoculation, longer gestation interval and lower physical activity in estrus time (1, 20, 21).

The provenance of the studied cows led to the hypothesis that independent animal factors such as the type of feed used, their administration and the maintenance system in shelters could be involved in the incidence of ketosis with a high frequency of puerperal pathology (Table 9). The predisposing factor in feed in

the appearance of ketosis is poorly fermented silage due to the high content of butyrate, so cows can not metabolize so quickly an increased intake of butyrate and resulting in the development of ketosis (11). Therefore, there is no significant relationship between farms and the determined variables, therefore the appearance of ketosis did not attract attention from the perspective of farm management.

Table 9

The ratio between the two groups (control and control) in terms of service period, level of beta-hydroxybutyrate concentration and milk protein fat ratio. (p <0.05)

Lot		Service p.	BHB	Report
Control	Mediate	1.361.250	. 2660	. 8144
	N	88	88	88
	Std. Deviation	48.79156	. 38083	. 67099
BLANK	Mean	85.2069	. 0779	. 3894
	N	87	87	87
	Std. Deviation	22.59921	. 12057	. 31195
Total	Mean	110.8114	. 1725	. 6031
	N	175	175	175
	Std. Deviation	45.76682	. 29765	. 56447

Conclusions

This paper presents a vision of the puerperal period from the perspective of the involvement of ketosis with negative consequences on the economic profitability of the farm and on the health of animals.

The quantification of the results obtained indicated that certain factors such as breed, farm of origin, season, cannot be directly attributed to the development of ketosis with consequences in the sphere of the reproductive system. Certainly the increased level of beta-hydroxybutyrate, an increased protein-fat ratio entails a higher number of artificial inseminations, a longer service period and losses of cows from the point of view of reproductive life.

However, the productivity of cows is constantly improving, which entails higher risks in terms of health. Overall, the productivity of animals is normal to tend to improvement, but is directly proportional to the risk of developing some pathologies. Thus, risk minimization must be performed constantly by monitoring metabolic parameters and early diagnosis of the disease.

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DIAGNOSTIC IMAGING AND CLASSIFICATION OF PORTOSYSTEMIC SHUNTS IN DOGS

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Summary

Portosystemic shunts affect the physiological trajectory of splanchnic blood in the gastrointestinal tract and abdominal organs, draining it directly into the systemic circulation. Consequently, portal blood bypasses the liver and is not subject to hepatic metabolism, making intra- and extrahepatic shunts an abnormal vascular connection (4). The most common types of shunts in veterinary medicine are represented by extrahepatic portosystemic, intrahepatic, and multiple acquired portosystemic shunts. Recent studies described breed predisposition and a genetical, inherited base of shunts component with no sex predilection: intrahepatic shunts most common seen in large breeds of dogs (Irish Wolfhounds, Golden Retrievers, Labrador Retrievers, Australian Cattle Dogs, Old English Sheepdogs) and extrahepatic shunts observed in small breeds such as Cairn Terrier, Yorkshire Terrier, Russell Terrier, Dachshund, Schnauzer, Bichon Maltese. (1, 5-17, 21-23). Multiple acquired extrahepatic shunts form secondary to portal hypertension, often due to primary hepatic parenchymal disease. There are a variety of shunts with portal and periportal starting point, including gastrophrenic, pancreaticoduodenal, splenorenal, mesenteric, and hemorrhoidal collateral vessels. The classification of the systemic shunts is a continuous and dynamic process with multiple changes and new connections found every year. Imaging diagnostic methods followed a gradual transition from ultrasound to CT, computed tomography being able to describe the complexities of shunts providing a detailed assessment of the abdominal blood vessels and a better understanding of the abnormal connections. The aim of the article is to describe and review the most common types of shunts and their pathophysiological implications.

Keywords: Portosystemic shunts, ultrasound, CT, dogs.

Knowledge of congenital anomalies of the portal venous system is essential for proper diagnosis and awareness of anatomic variants is crucial for adequate planning of surgical and interventional procedures and may also prevent significant complications (12).

Variants of portal branches and intrahepatic portosystemic shunts are quite uncommon; however, when present, they should be recognized before performing surgery or interventional procedures (12).

The first report of these malformations known as "Abernethy Syndrome" was made in 1973 by a London surgeon, John Abernethy, who described after a post-mortem examination of a 10-month-old child, the completion of the portal vein in the

inferior vena cava, in the renal veins. Congenital portosystemic venous shunts are rare and have been explained by changes in the embryological development of the portal and caval systems, with abnormal involution of the vitelline veins and may be associated with other congenital malformations: septal, patent arterial canal and Fallot tetralogy, biliary system-congenital biliary atresia, genito-urinary system-cystic kidney dysplasia and skeletal system-radial hypoplasia.

Congenital extrahepatic shunts represent the abnormal functionality of communication between the embryonic vitelline veins, which form the entire extrahepatic portal system, and the cardinal venous system, which normally contributes to the formation of non-portal abdominal veins. The extrahepatic portal vein develops from different parts of the vitelline vein, and the vena cava and the (hemi) azygos vein develop from the embryonic cardinal vein. The connections between the cardinal and vitelline systems do not occur at any stage of embryonic development. Therefore, extrahepatic shunts are considered abnormalities of vascular development.

Normally anatomical and functional main portal vein is formed by the confluence of the cranial and caudal mesenteric veins and the splenic vein, and therefore drains most of the blood from the abdominal organs into the liver (5, 7). The portal vein also receives a smaller tributary vein, the gastroduodenal vein, a few centimetres caudal to the hepatic entrance and connects to the right-ventral aspect of the portal vein after draining the pancreaticoduodenal and gastroepiploic veins. The splenic vein is joining the main portal vein on the left side, which follows the left limb of the pancreas after receiving the left gastric vein. The caudal extension of the portal vein is represented by the cranial mesenteric vein that receives all the jejunal branches (7). The diameter of the portal tributaries becomes enlarged as they approach the main portal vein, and the flow is directed towards the liver, the flow being known as the hepatopetal flow (15, 19).

Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) combined with Doppler.

Ultrasonography (US) permits a comprehensive evaluation of morphologic and functional abnormalities of the portal system (Fig. 1). Angiography has got nowadays a diagnostic value only for a pathophysiological evaluation for hepatic and portal vein pressure measurement while its role remains important in cases in which treatment is considered necessary such as TIPS positioning and portal vein embolization, used mostly in human medicine (12).

For assessment of the abdominal vasculature by computed tomography, a dual phase, low-pitched imaging protocol with good respiration control is used for an appropriate vascular study.

The liver parenchyma shows a homogeneous density in the range of about 60 – 70 HU in dog, values depending on technical settings (kVp, mA, slice width) and the enhancement is intense and homogeneous after contrast medium administration. Contrast resolution is the capacity of a system to accurately represent differences in tissue, physical, and/or biochemical characteristics, which

are intrinsically linked to x-ray attenuation (22-23). The intrahepatic vasculature includes an arterial supply and double venous system-hepatic and portal veins (22, 23). In dual-phase CT angiography, arterial and venous vessels can be identified (1, 2, 18). The largest hepatic veins are often visible already before contrast administration as hypodense tubular structures, becoming more evident in the arterial and venous phase of contrast administration.

Intrahepatic portal vasculature (Fig. 2) can be distinguished in CT during the venous phase following the division of the main portal vein into the two main right and left branches which supply the different liver lobes. For a better spatial representation of the hepatic vessels, maximum intensity projection and 3-D reconstructions can be used (22).

On a CT scan, portal vein and collaterals can be seen running toward the periphery, having low density in pre-contrast study and in arterial phase, being highly attenuating in the venous phase.

Ultrasound and CT scan are used mainly in veterinary medicine for the diagnostic of the vascular malformation. Ultrasound evaluation of the portal vein and abdominal vasculature is used as a first line investigation option in case of primary pathology of the portal vein, blood flow, velocity, and abnormal connections, but also in different cases of abdominal diseases which are indirectly related with the periportal space and portal vein. Computed tomography represents an advance imaging approach of abdominal pathology due to its excellent morphological resolution and its ability to image many different structures.

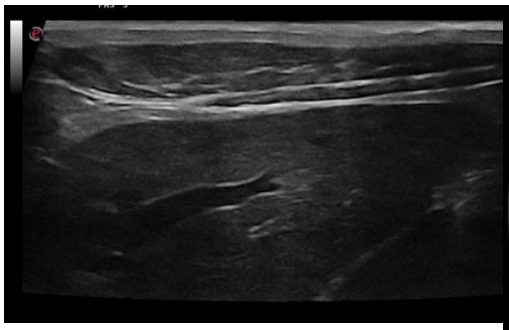

Fig. 1. Left branch of a normal portal vein - Normal appearance of the portal vein and portal branches is seen as a tubular structure with hyperechoic walls passing within the liver parenchyma

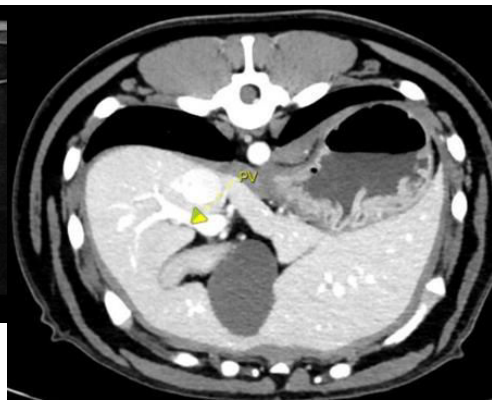

Fig. 2. Normal CT appearance of the portal vein at the level of porta hepatis in transverse section (yellow arrow)

Materials and methods

The present study was performed to describe, analyze, and classify portosystemic shunts, intra-, extra-hepatic and multiple acquired.

Confirmed cases of portosystemic shunts diagnosed by abdominal CT and ultrasound scan have been analyzed retrospectively in the St. George's Veterinary Hospital (Wolverhampton, UK) between January 2018 and March 2021. Triphasic abdominal CT (aortic, portal and delayed-venous), with slices between 0.2 and 0.75 mm (SOMATON Scope, 16 slice CT, Siemens, USA), have been performed, results being recorded and analyzed. General anesthesia has been used and intravenous fluids have been administrated pre and post examination. Complete blood work has been done for all the patients included in the study before undergoing CT examination. A positive non-ionic iodinated contrast agent consisting of iohexol (Omnipaque™, GE Healthcare) was administered via an intravenous bolus, according to the literature and a dual phase CT angiography: arterial phase (9-16 s): cranial to caudal and a venous phase (10-35s): caudal to cranial have been completed (21- 23). The abdominal ultrasound cases evaluated for liver pathology have been reviewed and data recorded for the present study. For the abdominal ultrasound examination, an ultrasound Esaote MyLab™Seven (Esaote, UK) device was used, with 2 transducers, convex and linear, with frequency adjusted according to the patient's size, clinical presentation, and age.

From the cases, diagnostic of vascular shunts malformation has been made in 24 cases.

The dogs included in the study (24) were represented mostly by small or toy breeds weighing between 1 –9.5 kg (18) and 6 medium size dogs with weight between 15-30 kg. CT angiography and abdominal ultrasound was used in all the cases for the diagnosis of PSS and further description of the velocity in cases of suspected portal hypertension. Diagnosis has been represented by: dogs with single extrahepatic PSS (17) - (Fig. 3, 4, 5): Single extrahepatic gastroduodenal (3), right gastrocaval (8), portocaval (4), splenocaval, (1), left gastrophrenic (1). 5 dogs have been diagnosed with intrahepatic shunt (Fig. 6) and 2 patients presented MAPSS – (Fig. 7). Breeds included in the study: Yorkshire Terrier (11): 8 males and 3 females; Ccrossbreed (7): 4 males and 3 females; Shih Tzu (4), 2 males and 2 females; 1 female Saluki, 1 Labrador male, with ages between 3 months and 9 years.

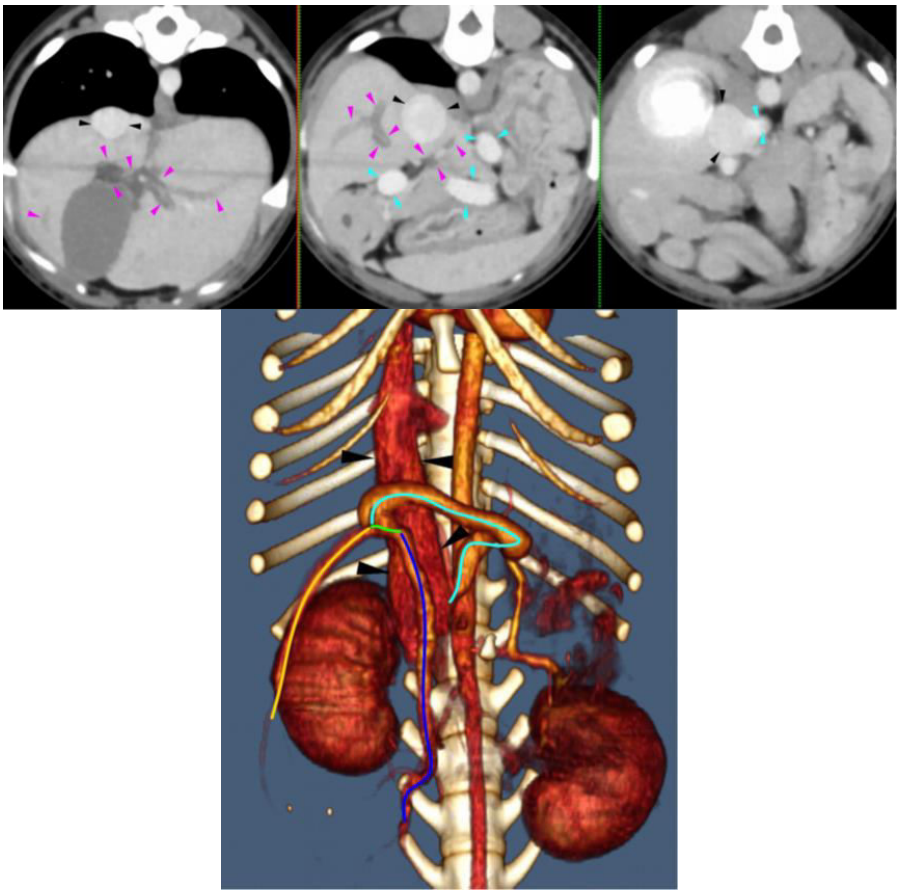

Fig. 3. Congenital extrahepatic right gastrocaval portosystemic shunt

A large (up to 6 mm), tortuous vessel (cyan arrowheads and line) arising from the portal vein at the level of the junction between the gastroduodenal (green line) and pancreaticoduodenal (yellow line) veins and running along the lesser curvature of the stomach. It enters into the left aspect of the caudal vena cava (black arrowheads) more caudally than its origin, at the level of the cranial pole of the right kidney. The portal vein (blue line) cranial to the shunt is very small (1.2 mm vs 3.7 mm before the shunt) but branches normally into the liver. A halo of fluid attenuation surrounds the visible intrahepatic portal branches as well as the region of the other intrahepatic portal branches (magenta arrowheads)

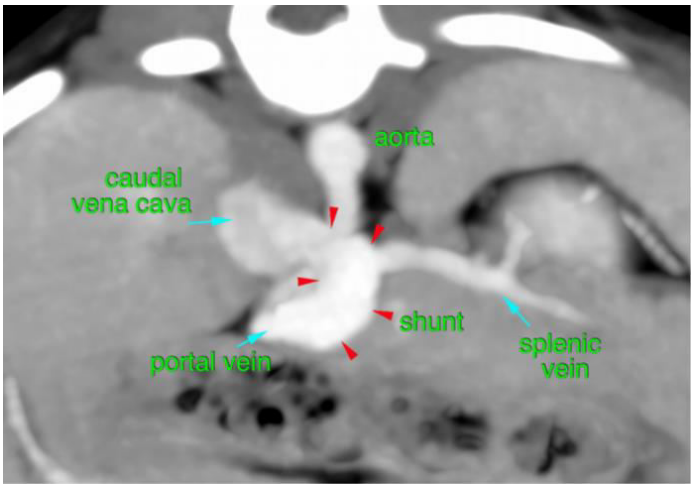

Fig. 4. Congenital extrahepatic portosystemic shunt - microhepatia

A short and curved anomalous vessel (red arrowheads below) cranial to spleno-portal communication courses between the portal vein and the caudal vena cava. This vessel is left sided, and partially communicates with the distal splenic vein. It measures 6.5 mm in diameter at the level where it joins the caudal vena cava. Further cranial to this site, the portal vein is abruptly thinned

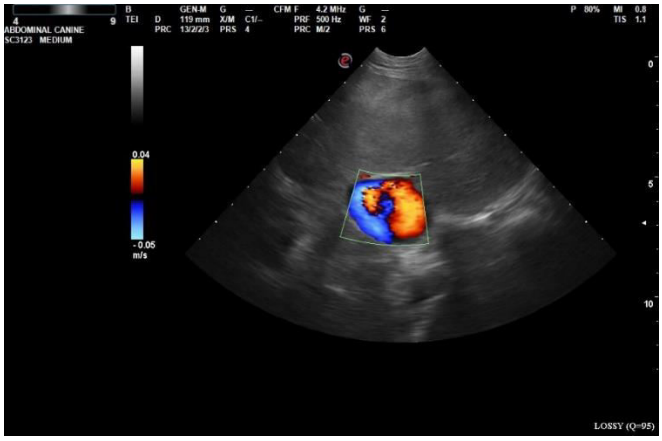

Fig. 5. Ying-Yang sign

Focal dilation of the abdominal vena cava seen in a dog with hyperechoic and diffusely enlarged lived, peritoneal collection, portal hypertension and portocaval shunt.

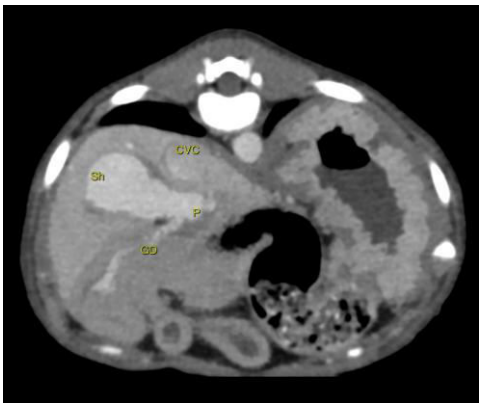

Fig. 6. Right divisional shunt

Is represented by a large right turn of a vessel in direct continuity with the portal vein, making a hairpin turn in the right and rapidly reaching the caudal vena cava. The shunting vessel measures a maximum of 16 mm in diameter. At its junction with the caudal vena cava, the diameter is about 15mm. There is reduction in volume of the liver and mild periportal halo. Junction of the shunting vessel is caudal to the junction of the left hepatic veins with the caudal vena cava.

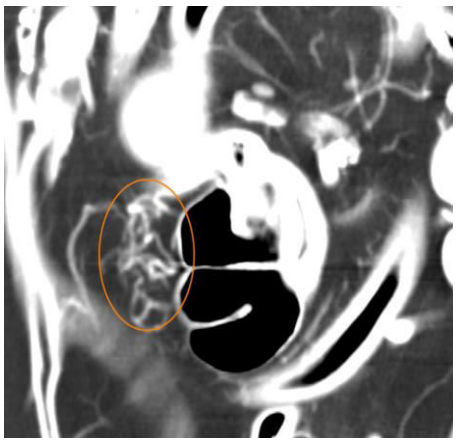

Fig. 7. Multiple acquired portosystemic shunts.

Plexus caudolateral to the left kidney connecting to left renal vein. Smaller plexus caudal to the right kidney. A vascular structure extending to the caudal aspect of the left renal vein, caudally and to the lateral aspect of the kidney joins a plexus of vasculature that connects to the splenic (portal) vasculature). The left renal vein is ~2X the size of the right. A smaller plexus of vasculature is noted caudal to the right kidney, that cannot be discretely connected to the portal system.

Results and discussions

Multiple classifications of the intrahepatic and extrahepatic shunts have been made in multiple studies (1, 2, 3, 14, 15, 16, 17, 21, 22, 23).

A recent study of the intrahepatic portosystemic shunts classifies them as**:** Right divisional, left divisional, central divisional, but also described a sub classification depending on the insertion, later one being useful in additional description of the morphological variation and possible more accurate surgical planning (16).

New classification proposal has been made for intrahepatic portosystemic shunt in dogs:

- Persistent ductus venosus (left, right, central)
- Aneurysmal IHPPS
- One or more PSS in a single liver lobe
- Multiple PSS in several liver lobes

In a study, 92% of IHPSS were observed inserting into the CVC via a primary HV or phrenic vein. Identifying which hepatic venous structure an IHPSS involves can promote consistent and accurate classification of the IHPSS as right, left, or central divisional. Classification of the IHPSS based on the venous structure it inserts through is likely to be more reliable than attempting to classify the IHPSS based on the portal vasculature, since the intrahepatic portal branches are often small or not visible on CTA in dogs with IHPSS (16). Also, in the same study has been prove that since the hepatic parenchyma may be asymmetrically hypoplastic, resulting in lateral shift of normal anatomical structures, such as the gallbladder and the portal vein itself, to one side, is making the traditional characterization further unreliable. Same principle applies in case of not proper distinction of the hepatic lobes with CTA. The position of insertion of the hepatic veins into the CVC will remain constant despite these potential shifts, allowing easy distinction with CTA, providing a consistent method of IHPSS classification. Subclassification of left, central, and right divisional IHPSS allows additional description of the morphological variation within these categories (1, 2, 3, 15, 16).

Extrahepatic portosystemic shunts (EHPSSs) affect the physiological trajectory of splanchnic blood from the gastrointestinal tract and abdominal organs, draining it directly into the systemic circulation. There are multiple types of extrahepatic portosystemic shunts, and the classification can be made depending on the abnormal connections and origin of the blood vessels. One of the descriptions of the EHPPS it is made as type one or end-to-side EHPSS and type two or side-to-side. These different types have different clinical impact, treatment options and outcome. In the type I or end-to-side EHPSS the connection is directly between the portal vein and a systemic vein due to CAPV and there is no discernible portal flow to the liver.

In type I or end-to-side extrahepatic portosystemic shunts, the connection is direct between the portal vein and a systemic vein and there is no portal flow to the

liver. The type II or lateral shunt includes a vascular connection between a portal tributary, most commonly from the left or right gastric veins, and a systemic vein (cava or azygos vein). These connections most often come from the left or right gastric veins. Side-to-side is the most common type of congenital portosystemic shunt described in veterinary patients (11, 12). Various repetitive phenotypes of side-to-side EHPSS, which reflect a common underlying embryological error, have been described in dog and cats (1, 2, 3).

Anomalous portosystemic connections can also involve the mesenteric tributaries of the portal vein system, as previously reported: mesenterico-renal-caval shunt has been reported in dogs and left colic vein or cranial rectal vein to pelvic systemic vein communications (directly to the caudal vena cava or through common iliac vein or internal iliac vein) (17).

In side-to-side EHPSS, there is preservation of at least some hepatic portal flow (4).

Many of these patients have extremely hypoplastic portal vein cranial to the shunt that is sometimes difficult to visualize with conventional CT angiography. High quality, thin-slices MDCT images may help in detection of a thin portal vein and portal branches.

Multiple types and combinations of extrahepatic portosystemic shunts have been described: splenocaval, portocaval, splenophrenic, splenoazygos, right gastro-caval and right gastrocaval with caudal location of the shunt. The left or right gastric veins can emanate shunts joining the caudal vena cava, phrenic vein, or the azygos vein directly or can connect with left spleno-gastric veins and then enter the systemic vasculature, seen also in the present study. Caudal tributaries contributing to the portal venous system, mesenteric and colic veins can connect directly to the caudal vena cava or enter the systemic circulation via renal vein or pelvic veins.

Additional variations in shunt anatomy that do not conform to this general classification can be observed. A description of the shunts involving the left gastric vein identified variants that entered the phrenic vein, caudal vena cava and azygos vein. Multiplanar reforming and 3D rendering can be useful in defining anatomy (21).

These patients can be treated using partial shunt banding to allow gradual expansion of the intrahepatic portal system while avoiding irreversible portal hypertension. Splenosystemic shunts with the azygous vein, the left phrenic and left gonadal veins have been also reported, but their definitive aetiology is still debated (1, 2, 3, 8, 12, 13, 14).

Acquired Portosystemic Shunts (APSSs):

APSSs are characterized by hepatofugal pathways that can be caused by portal hypertension or increased resistance in the cranial vena cava system leading at reopening of the previous vascular connections between the portal and systemic circulation (6).

Multiple patterns of APSS have been reported in dogs and cats (2, 20, 21).

They can be simply divided depending on the size of the blood vessels in large shunts (left splenogonadal or splenophrenic shunt) and small shunts

(oesophageal, gastrophrenic or colic varices). Both types of shunts can often coexist in the same patient (2). As previously mentioned with the rest of the shunts, MAPSS can be also described depending on their anatomical locations and connections.

These are best detected by scanning the entire abdomen and using a thin collimation for maximum spatial resolution. Small collateral vessels may have a lower resolution compared to individual vessels, depending on the limitation of spatial resolution.

Porto-systemic shunts are relatively common in dogs, and the most common types of shunts are single extrahepatic in small breed dogs and unique intrahepatic in large breeds. Intrahepatic portosystemic shunts are usually of congenital origin, while extrahepatic shunts may be congenital or secondary to some form of portal hypertension. Most congenital extrahepatic portosystemic shunts originate in the portal vein, but in most cases involve the splenic vein or right gastric vein, described in multiple studies and as well as in the present one. Intrahepatic portosystemic shunts (IHPSS), more common in medium and large breed dogs and are traditionally classified as left, central, or right division, based on the hepatic lobe through which they pass. In the present study, intrahepatic shunts, have been seen in dogs with weight between 15-30 kg and after the age of 8 months. The classification of the IHPSS in relation with the draining site and connection with local vasculature is more reliable in order of an accurate diagnostic and surgical planning. Extrahepatic portosystemic shunts have been seen mostly in toy breeds, Yorkshire Terrier being the most representative one in this study, clinical signs starting as early as 3 months, clinical signs being represented mostly by neurological ones. In examination of the portal venous system, the scanning direction should always be selected cranio-caudally to avoid the artifact caused by diaphragmatic movements secondary to the respiratory phase. In the case of CT angiography and the detection of possible vascular malformations, a cross-section on the abdomen is preferable, which provides a complex overview. The classification of portosystemic shunts is a dynamic process, and the diagnosis must be made according to the vascular insertion, the origin of the shunt and their possible communications at the abdominal level. Other imaging findings represented by microhepatica, lithiasis, turbulent flow (seen in a dog with potocaval shunt as well as periportal hyperechoic cuffing) can also be present in cases of portosystemic shunts.

Conclusions

PSS classification is an evolving process, as more and more types of shunts and combinations are identified by advanced imaging methods. Porto-systemic shunts are relatively common in dogs, and the most common types of shunts are single extrahepatic in small breed dogs and unique intrahepatic in large breeds, seen also in the present study. Intrahepatic portosystemic shunts are usually of congenital origin, while extrahepatic shunts may be congenital or secondary to some form of portal hypertension.

Most congenital extrahepatic portosystemic shunts originate in the portal vein, but in most cases involve the splenic vein or right gastric vein, described in multiple studies and as well as in the present one. The splenic vein may be present with multiple shunt conformations. Highlighting the shunt at the level of the right gastric vein is sometimes difficult to confirm, adjuvant methods being the examination of the small curvature of the stomach (usual location of the right gastric vein) and 3D reforming.

Intrahepatic portosystemic shunts (IHPSS) are more common in medium and large breed dogs are traditionally classified as left, central, or right division, based on the hepatic lobe through which they pass. In the present study, intrahepatic shunts, have been seen in dogs with weight between 15-30 kg and after the age of 8 months. Extrahepatic portosystemic shunts have been seen mostly in toy breeds, Yorkshire Terrier being the most representative one in this study, clinical signs started as early as 3 months.

In the examination of the portal venous system, the scanning direction should always be selected cranio-caudally to avoid the artifact caused by diaphragmatic movements secondary to the respiratory phase.

In the case of CT angiography and the detection of possible vascular malformations, a cross-section on the abdomen is preferable, which provides a complex overview. Congenital intrahepatic and extrahepatic portosystemic shunts are well seen, and their complex anatomy can be characterized using 3D reconstructions. Key elements in this examination include establishing the correct delay in initiating the scan in relation to the administration of contrast and an adequate collimation of the image in relation to the size of the patient.

Multislice CT improves image quality and allows better synchronization of vascular phases. Multislice CT can obtain images in both arterial, venous-portal and delayed phase with low collimation and can more easily highlight venous abnormalities and their trajectory, insertion and contrast intake, elements used for a better classification of the shunts.

Common elements highlighted by CT examination in the description of portal vascular abnormalities are represented by the diameter of the portal vein decreases cranially at the outlet of the shunted vessel; abnormal vessels are often large when they arise from the splenic vein and small when they arise from other veins, with a sinuous path between the portal circulation and the systemic circulation as previously described. Other imaging signs are represented by microhepatica, peritoneal effusion, urolithiasis, nephrolithiasis, and any other congenital anomalies. In the case of portosystemic shunts ending at the diaphragmatic level, due to respiratory compression, depending on the respiratory cycle, some patients do not show clinical signs characteristic of shunts, since the blood is shunted only at the time of expiration. These patients may have normal-sized liver and no other clinical signs. This pathophysiological abnormality is recorded especially in the case of splenophrenic shunts.

Hyperechoic periportal cuffing and turbulent flow (Fig.5) seen on the abdominal ultrasound and periportal halo sign (CT) can be present in cases of portosystemic shunts (9), seen mostly with portocaval shunts. A periportal cuffing ultrasound and a hyperechoic appearance of the portal vasculature in absence of any other major abdominal ultrasound findings, it should guide us to a different approach of the examination, portal hypertension, primary heart disease, congenital processes and vascular abnormalities being involved in the systemic changes of the portal vessels ultrasonographic aspect (10).

Multiple acquired extrahepatic shunts form secondary to portal hypertension, often due to primary hepatic parenchymal disease. In those cases, a pseudoaneurysm of the portal vein can be seen as well as hyperechoic blood vessel walls due to vasculitis and increased blood pressure. There are a variety of shunts with portal and periportal starting point, including gastrophrenic, pancreaticoduodenal, splenorenal, mesenteric, and hemorrhoidal collateral vessels.

Although the genetic basis of congenital portosystemic shunts in dogs has not been clearly established, many studies have shown that this condition occurs more frequently in purebred dogs and is inherited in several small breeds (21). Even though a sex predisposition has not been fully researched, in the present study, males have been more affected by vascular malformations. The article aims to present portosystemic shunts imaging description in dogs (in relation with the insertion point, caliber of the vessel, local anatomy, position, contrast intake) and general clinical picture that needs to be made for full assessment and review literature and studies involving the portosystemic vascular malformations.

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PREVALENT AEROBIC BACTERIAL FLORA ISOLATED FROM EMBRYONATED EGGS AND NON-VIABLE PHEASANT CHICKS

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Summary

The study aimed to identify aerobic bacteria isolated from pheasant eggs incubated in the hatchery incubator, as well as from pheasant chicks that died on the first day of life. 323 hatching eggs and 101 one-day-old non-viable pheasant chicks were studied for the repopulation of game funds: common pheasant (*Phasianus colchius colchius*) and black pheasant (*Phasianus colchius varietas tenebous*). From the pathological material examined, it was observed that a pathogenic conditioned aerobic bacterial flora predominated: *E. coli, Pseudomonas, Staphylococcus aureus* and *Proteus vulgaris*. Sensitivity to various antimicrobial substances was tested for isolated strains.

Keywords: pheasant eggs, *E. coli, Pseudomonas, S. aureus*, *Proteus.*

The operation of repopulating the hunting parks is done by incubating the pheasant eggs from their breeding birds that are housed in outdoor pens. During the laying of eggs, they can become contaminated, sometimes the source of infection being the incubators, improperly disinfected. The typical hatching for this operation is about 60-75% (± 3%). The causes of low hatchability were or the death of embryos in the late stage of development can be multiple, but these are added bacterial infections. Some chicken embryos pass through the shell but may die before hatching. The outer surfaces of unhatched eggshells facing low hatching should be subjected to bacteriological evaluation. Embryos from unhatched eggs should be removed aseptically from eggshells and subjected to bacteriological evaluation.

A major danger that affects the life of embryos is represented by pathogens responsible for the onset of infectious diseases which can be transmitted directly from sick birds or birds that have passed through the disease to embryos through the germline of eggs (3, 5, 10, 13, 24).

The paper aimed at the aerobic bacterial flora of pheasant eggs, which can influence the hatching percentage, but also the pathogenicity of the isolated and identified germs.

Materials and methods

The study included: 323 hatching eggs and 101 non-viable eggs of common pheasant (*Phasianus colchicus colchicus*) and black pheasant (*Phasianus colchicus tenebrous variety*) used to repopulate game funds. Bacteriological examination and antibiogram were performed. The embryo egg samples were properly asepticized, placed in a plastic container and transported to the laboratory for bacteriological examination. To avoid contamination of the egg contents with the germs found on the shell, it was wiped with a sterile swab, with normal saline (0.85%), and finally, the surface was sterilized by a rapid passage through the flame (1, 2, 7, 23, 25).

The contents of the samples to be examined were aspirated with a sterile Pasteur pipette and placed in a culture medium. From each sample, bacteriological examinations were performed on common and special culture media.

Bacteriological examination was performed in nutrient broth for bacterial growth and then on nutrient agar, MacConkey agar, blood agar, xylose-lysine deoxycholate agar (XLD), SS. agar, Levine agar and Chapman agar. Based on the cultural and morphological characteristics, the identification of isolated bacteria was performed. The pathogens were isolated and identified by culturing and incubation for 24 hours at 37° C. During the morphological examination of the isolated bacteria, the following aspects were followed: their macroscopic and microscopic morphology, size, shape, grouping, colour and colour change on different culture media. To confirm the diagnosis, biochemical tests were performed, including coagulase, catalase, oxidase, MIU medium, TSI medium. The definitive identification of the isolated etiological agents was made with diagnostic kits: API 20 E - for the identification of pathogens from the *Enterobacteriaceae* family, API Staph - for the identification of staphylococci (API - Analytical Profile Index) (24, 27, 28).

The antimicrobial susceptibility of the isolated strains was tested by the diffusimetric disk method (Kirby-Bauer method), using the Muller-Hinton medium and antibiotic-impregnated discs, by the manufacturing companies. The following antimicrobial substances were used to test the behaviour of *E. coli* strains: amoxicillin, colistin, doxycycline, enrofloxacin, florfenicol, gentamicin, lincospectin, neomycin, trimethoprim, and tetracycline.

Staphylococcus spp. strains were tested against the following antimicrobials: ampicillin, amoxicillin, colistin, enrofloxacin, gentamicin, kanamycin, florfenicol, trimethoprim, tetracycline and streptomycin.

Pseudomonas spp. strains were tested to the following antimicrobials: amoxicillin, doxycycline, erythromycin, enrofloxacin, florfenicol, gentamicin, lincospectin, neomycin, tetracycline, trimethoprim.

The placement of the antimicrobial substances was carried out at a distance of 20-30 millimetres from each other and at a distance of 15 millimetres from the edge of the plate. The results of the antibiogram reading were interpreted according to the European Committee for Antimicrobial Sensitivity Testing (EUCAST). The diameter of the inhibition zone was measured with a ruler and expressed in

millimetres. Depending on the diameter read, the results were classified as sensitive, intermediate, and resistant (24, 27, 28).

Results and discussions

From the pathological material examined, it was observed that a pathogenic conditioned aerobic bacterial flora predominated: *E. coli, Pseudomonas, Staphylococcus aureus* and *Proteus vulgaris* (Table 1, Fig. 1). Sensitivity to various antimicrobial substances was tested for isolated strains.

Table 1

Frequency of bacteria isolated from embryonated eggs and non-viable pheasant chicks

	Isolated bacteria									
Samples	No. exams	No. isolated	*E. coli*		*Pseudomonas spp*		*Staphylococcus spp*		*Proteus*	
			No.	%	No.	%	No.	%	No.	%
Pheasant embryonated eggs	323	110	58	18	26	8	23	7.12	3	0.92
Non-viable pheasant chicks	101	13	6	6.06	2	2.02	3	4.04	2	2.02

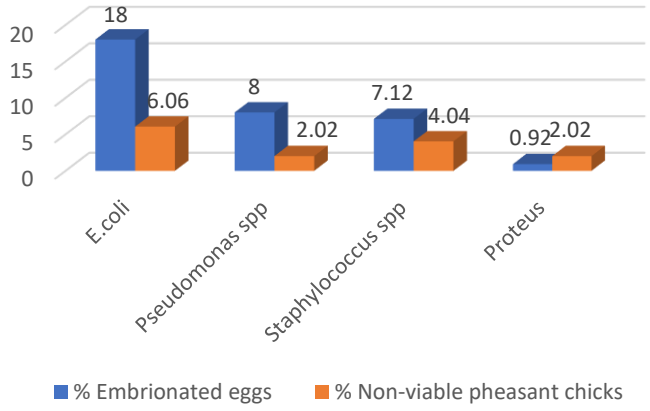

Fig. 1. Distribution of isolated bacteria

Out of 323 embryonated eggs, 110 strains were isolated, of which: 58 strains of *E. coli*, 26 strains of *Pseudomonas spp*, 23 strains of *Staphylococcus* spp. and 3 strains of *Proteu*s.

In the case of the 101 non-viable pheasant chicks, 13 strains were isolated: 6 strains of *E. coli*, 2 strains of *Pseudomonas spp*., 3 strains of *Staphylococcus spp*., and 2 strains of *Proteus*.

Bacterial flora isolated from embryonated eggs showed that the predominant species were: *E. coli* 18 %, *Pseudomonas 8%, Staphylococcus* spp. 7.12%, *Proteus* 0.92% and non-viable day-old chicks *E. coli* 6.06 %, Staphylococcus spp. 4.04%, Pseudomonas spp. 2.02% and *Proteus vulgaris 2.02%.*

Bacterioscopic examination revealed Gram-positive as well as Gram-negative bacteria.

The isolated strains had a characteristic behaviour on the culture media, which allowed the classification in the respective genus. The final identification of *Enterobacteriaceae* and staphylococcus strains was made using the API 20 E kit and the Staph API kit.

From 64 *E. coli* strains isolated from non-viable embryonated pheasant eggs and non-viable pheasant chicks, antibiotic susceptibility was tested to 24 strains (Table 2, Fig. 2). They were sensitive to florfenicol (66.67%), enrofloxacin (62.50%), gentamicin (54.17%), trimethoprim (45.83%), colistin (37.50%), amoxicillin (29.17%). Lincospectin, tetracycline and neomycin were less active.

The highest resistance of the tested *E. coli* strains was 100% doxycycline, neomycin and tetracycline (70.83%).

From 28 isolated *Pseudomonas spp*. strains, 24 strains were tested to the antimicrobials substances (Table 3, Fig. 3).

From the data obtained it can be seen that the isolated strains of *Pseudomonas spp*. were sensitive to gentamicin (91.67%), florfenicol (70.83%), enrofloxacin (66.67%). Most strains were resistant to tetracycline, trimethoprim, doxycycline, erythromycin (100%), amoxicillin (95.83%), neomycin (87.5%) and lincospectin (75%).

From 26 isolated strains of *Staphylococcus spp*., 24 strains were tested to 10 antimicrobial substances: gentamicin, kanamycin, florfenicol, trimethoprim, colistin, amoxicillin, tetracycline, enrofloxacin, streptomycin and ampicillin (Table 4, Fig. 4).

The 24 strains of *Staphylococcus spp*. tested were sensitive: ampicillin (75.00%), amoxicillin (79.17%), florfenicol (70.83%), kanamycin (62.50%), and gentamicin (66.67%). They were intermediate sensitive to trimethoprim (54.17%), streptomycin (45.83%) gentamicin, enrofloxacin, kanamycin, tetracycline (20.83%), colistin, florfenicol (16.67%).

Staphylococcus spp. strains showed multiple resistance to tetracycline (62.50%), colistin (54.17%), enrofloxacin (45.83%), streptomycin (41.67%), ampicillin and kanamycin (16.67%), florfenicol and gentamicin (12.50%), amoxicillin, (8.33%).

Table 2

Synoptic of antibiotic susceptibility of 24 isolates of *E coli*

	Sensible		Intermediate		Resistant	
Antibiotics	No.	%	No.	%	No.	%
Amoxicillin	7	29.17	11	45.83	6	25.00
Colistin	9	37.50	5	20.83	10	41.67
Doxycycline	0	0.00	0	0.00	24	100.00
Enrofloxacin	15	62.50	4	16.67	5	20.83
Florfenicol	16	66.67	5	20.83	3	12.50
Gentamicin	13	54.17	8	33.33	3	12.50
Lincospectin	5	20.83	14	58.33	5	20.83
Neomycin	2	8.33	5	20.83	17	70.83
Tetracycline	1	4.17	6	25.00	17	70.83
Trimethoprim	11	45.83	8	33.33	5	20.83

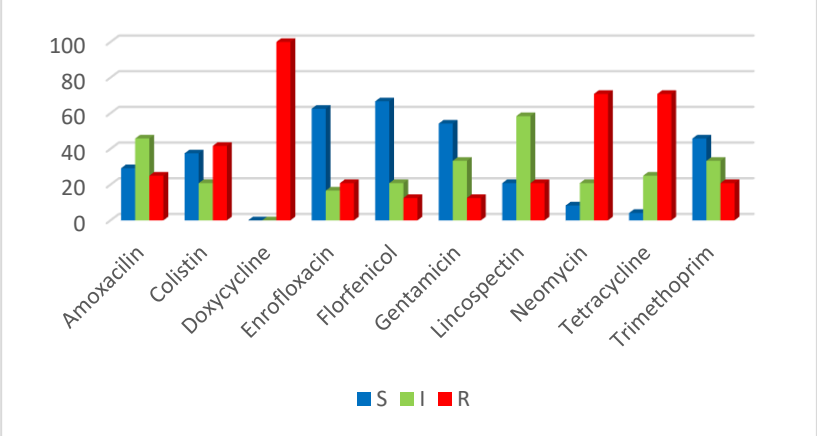

Fig. 2. Dynamics of antibiotic susceptibility of 24 *E. coli* isolates

Table 3

Synoptic of antibiotic susceptibility of 24 isolates of *Pseudomonas spp.*

	Sensible		Intermediate		Resistant	
Antibiotics	No.	%	No.	%	No.	%
Amoxicillin	0	0.00	1	4.17	23	95.83
Doxycycline	0	0.00	0	0.00	24	100.00
Erythromycin	0	0.00	0	0.00	24	100.00
Enrofloxacin	16	66.67	3	12.50	5	20.83
Florfenicol	17	70.83	5	20.83	2	8.33
Gentamicin	22	91.67	2	8.33	0	0.00
Lincospectin	0	0.00	6	25.00	18	75.00
Neomycin	0	0.00	3	12.50	21	87.50
Tetracycline	0	0.00	0	0.00	24	100.00
Trimethoprim	0	0.00	0	0.00	24	100.00

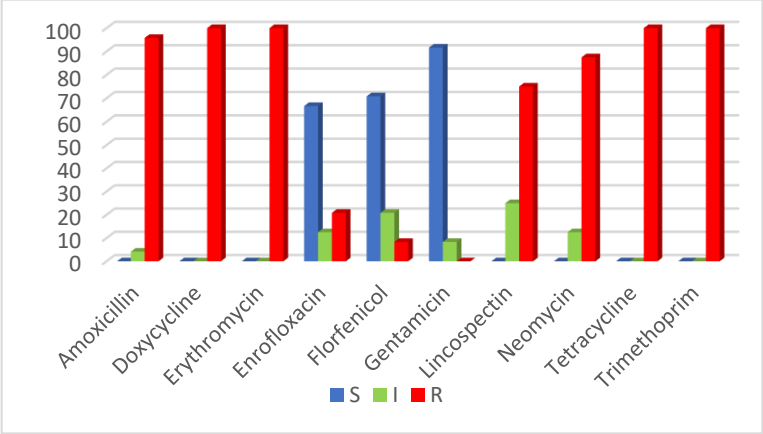

Fig. 3. Dynamics of antibiotic susceptibility of 24 *Pseudomonas* isolates

In a game farm, a low percentage of 14-15% hatching of pheasant eggs (*Phasianus colchium*) was recorded, with considerable losses. Bacteriology results did not identify pathogenic bacteria in the eggshells, embryo samples showed large amounts of *Enterococcus faecalis*. The only factor identified was an unusually warm period, followed by an atypically cold and wet period during egg collection for those eggs that are experiencing low hatching (15, 17, 22).

According to other authors, a total of 105 dead embryos in shell, randomly selected from three incubators was recorded as 9.91 % (8.04 to 12.03%). The following bacterial species were isolated: *Escherichia coli* 52.54%, *S. Gallinarum* 11.86% *S. Pullorum* 5.93%, *Streptococcus faecalis* 5.93%, *Bacillus subtilis* 4.2%, *Pseudomonas aeruginosa* 3.39% and *Proteus mirabilis* 3.39%. The in vitro sensitivity of the isolates to the 11 antibiotics was: norfloxacin 100%, gentamicin 89%, flumechin 80%, neomycin 68.64%, chloramphenicol 67.80%, streptopenicillin 65%, erythromycin 14.4% and furazolidone 14.4 % (3, 14, 18).

Table 4

Synoptic of antibiotic susceptibility of 24 isolates of *Staphylococcus spp*.

	Sensible		Intermediate		Resistant	
Antibiotic	No.	%	No.	%	No.	%
Ampicillin	18	75.00	2	8.33	4	16.67
Amoxicillin	19	79.17	3	12.50	2	8.33
Colistin	7	29.17	4	16.67	13	54.17
Enrofloxacin	8	33.33	5	20.83	11	45.83
Florfenicol	17	70.83	4	16.67	3	12.50
Gentamicin	16	66.67	5	20.83	3	12.50
Kanamycin	15	62.50	5	20.83	4	16.67
Streptomycin	3	12.50	11	45.83	10	41.67
Tetracyclin	4	16.67	5	20.83	15	62.50
Trimethoprim	5	20.83	13	54.17	6	25.00

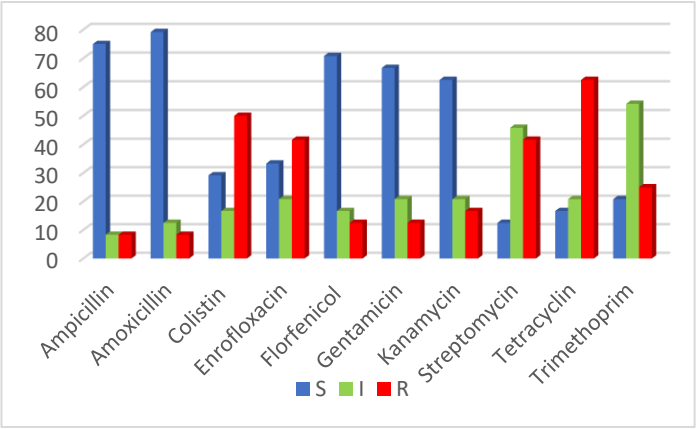

Fig. 4. Dynamics of antibiotic susceptibility of 24 *Staphylococcus spp.* Isolates

Excessive utilisation of antibiotics contributes to the development of multiple resistance. Other research reported that *E. coli* had the highest incidence (68.05%),

followed by *Enterobacter spp*. (16.67%), *Citrobacter spp*. (11.11%), *Klebsiella spp*. (3.47%) and *Bacillus spp*. (1, 2, 26).

In 121 chickens obtained from conventional eggs examined in the first 5 days of life, bacterial contamination of the peritoneal cavity of 38% was reported, infections of the yolk sac, 23%, the most common were streptococci, followed, in descending order, of micrococci and coliform organisms, especially E. coli (1, 4, 11, 12).

Other research includes a pathogenic conditioned aerobic bacterial flora represented by *Escherichia coli*, *Proteus vulgaris, Bacillus cereus, Staphylococcus aureus* and *Streptococcus spp.*, But also strains of *Salmonella djugu* (66.66%) and 33% *Salmonella typhimurium* (8, 9, 16).

Proteus vulgaris strains isolated from embryonated eggs were susceptible to kanamycin (78.78%), amoxicillin (78.78%), gentamicin (72.28%), furazolidone (56.07%), ampicillin (68) and resistance to the activity of tetracycline (53.02%) and chloramphenicol (48.50%) (24, 26).

In the case of *Escherichia coli* strains, they were sensitive to gentamicin (71.96%), kanamycin (66.10%), colistin (63.67%) and tetracycline-resistant furazolidone (52.43%), erythromycin (62.21%), streptomycin (57.33%) and ampicillin (52.43%) (24, 28).

Staphylococcus aureus strains were highly sensitive to gentamicin (97.62%), amoxicillin (95.24%), ampicillin (83.34%) and kanamycin (83.24%) resistant to streptomycin (64.48%) and 57.16% chloramphenicol (6).

At an Alabama chicken company, high mortality rates have been reported for chickens from two different hatcheries, where 5 strains of *Pseudomonas aeruginosa*, obtained from these contaminated incubators, were isolated (22) Antibiotic susceptibility testing showed that all five isolates were resistant to sulfisoxazole, ceftiofur, penicillin, lincomycin, bacitracin, oxytetracycline, erythromycin, nalidixic acid, and tetracycline, but all isolates were sensitive to gentamicin (19, 20, 21).

Conclusions

Bacterial flora isolated from embryonated eggs and non-viable chicks was represented by: *E. coli* 18%, *Pseudomonas* (8%), *Staphylococcus spp*. 7.12%, Proteus 2.2%.

E. coli strains were sensitive to: florphenicol (66.67%), enrofloxacin (62.50%), gentamicin (54.17%), trimethoprim (45.83%), colistin (37.50%), amoxicillin (29.17%) and were resistant to 100% doxycycline, neomycin and tetracycline (70.83%).

The isolated strains of *Pseudomonas spp*. were sensitive to gentamicin (91.67%), florfenicol (70.83%), enrofloxacin (66.67%) and resistant to tetracycline, trimethoprim, doxycycline, erythromycin (100%), amoxicillin (95.83%), neomycin (87.5%) and lincospectin (75%).

Staphylococcus spp. strains were sensitive to amoxicillin (75%), florfenicol (70.83%) gentamicin (66.67%) kanamycin (62.50%), and resistant to tetracycline (62.50%), colistin (54.17%), enrofloxacin (45.83%).

It is recommended to control the efficiency of disinfection in incubators as well as disinfection of eggs before incubation.

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DIAGNOSTIC TOOLS IN CANINE KIDNEY DISEASE. CASE REPORT

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Summary

Kidney disease in dogs is a very common condition in veterinary practice nowadays and can be extremely aggressive in both young and geriatric patients. Kidney disease, as a generically defines any renal lesion (of any size or degree) or clinic-pathological abnormality that pertains to renal function (Lattimer, K.S., 2011). It is known that kidney disease in dogs encompasses acute kidney injury (AKI), chronic kidney disease (CKD) and their primary conditions: neoplasia, glomerulopathies, etc. Diagnostic of a certain renal disease might be challenging because of nonspecific clinical and laboratory findings. Special attention should be paid to the anamnesis, physical examination and to the formulation of an individual well-established diagnostic plan in accordance with the available guidelines. Therefore, to accurately assess the extent of the lesion and loss of function, various diagnostic tools might be considered. These include non-invasive procedures: blood biochemistry (Creatinine, Urea, Calcium Phosphate, electrolytes), blood pressure measurements, urinalysis, (proteinuria, urine specific gravity, urine culture), diagnostic imaging, glomerular filtration rate (GFR) and other surrogate plasma markers of reduced GFR: Symmetric dimethylarginine (SDMA), completed by invasive procedures (renal biopsy) when necessary. This article aims to describe the principles of these tests and emphasize their usefulness in the diagnosis of canine kidney disease based on a case report.

Keywords: kidney, disease, diagnosis, urinalysis, dimethylarginine.

Cases of canine kidney disease are very frequently nowadays, and every veterinarian should know how to manage them. Even if there is a lot of information on this subject, a step-by-step diagnostic plan for canine kidney disease is always useful. Further characterization of the renal disease should include various diagnostic tools to rule out potentially treatable conditions/complications.

An example could be urine culture for urinary tract infection and kidney imaging for renal lymphosarcoma exclusion. In both CKD and AKI, the common clinical signs are nonspecific, and include lethargy, anorexia and vomiting. These clinical similarities at presentation, with presence of concurrent azotaemia in both conditions, can make the differentiation between these two conditions challenging. Differentiation is especially difficult when clear history and clinical signs of CKD are absent and recent Serum Creatinine value (SCr) is not available (9, 13).

Materials and methods

We have reviewed the available guidelines on this topic, and we have adapted them to our current facilities to establish a diagnostic plan for cases of suspected canine kidney disease. We have also documented the specific diagnostic tools and succinctly describe them here, emphasizing their usefulness basing on a clinical case of canine kidney disease. All the investigations were conducted in the Clinic of the Faculty of Veterinary Medicine Bucharest, excepting SDMA, UP/C and the histopathological exam which were performed in an authorized diagnostic center.

Results and discussions

Dogs with kidney disease may present a lot of non-specific clinical signs, which in absence of a thorough medical history can be difficult to categorize as AKI or CKD. In this article, we will present a case that may be of common presentation in any veterinary clinic but may become a challenging situation without a complete history (diet, vaccination and deworming status, previous disorders or treatments, toxic exposure).

Fig. 1. Mixed-breed dog, 9-year-old, female

Diana is an almost 9-year-old, female intact mixed-breed dog (Fig. 1), who came to the Internal Medicine Clinic with severe signs of lethargy, inappetence and dehydration. Medical history was almost absent because she is a stray dog, fed by a few people from the neighborhood. They remarked that she became lethargic and started to refuse food and water.

Physical examination revealed a low body condition score (2/5), lusterless hair coat, dry mucous membranes, normal refill capillary time, bad breath, mild dehydration, normal rectal temperature (38.3 C), tachycardia (126 bpm) and tachypnea (68 rpm), normal lymph nodes, nil abnormal palpable in abdomen, thorax auscultates normally.

Our first step in the management of this patient was to explore what we already know, more exactly to find out whether the azotemia was prerenal, renal, or postrenal. In this situation a thorough basic diagnostic plan (Fig. 2) is required to obtain more data about the patient's current medical condition and its determinant cause.

Mean systemic blood pressure was 141/95 mm Hg, which according to IRIS is categorized as prehypertensive stage. Blood samples were collected via the jugular vein and urine was collected via cystocentesis. After this an abdominal ultrasonography was performed to evaluate the urinary tract, the size, shape, and parenchymal architecture of the kidneys (8).

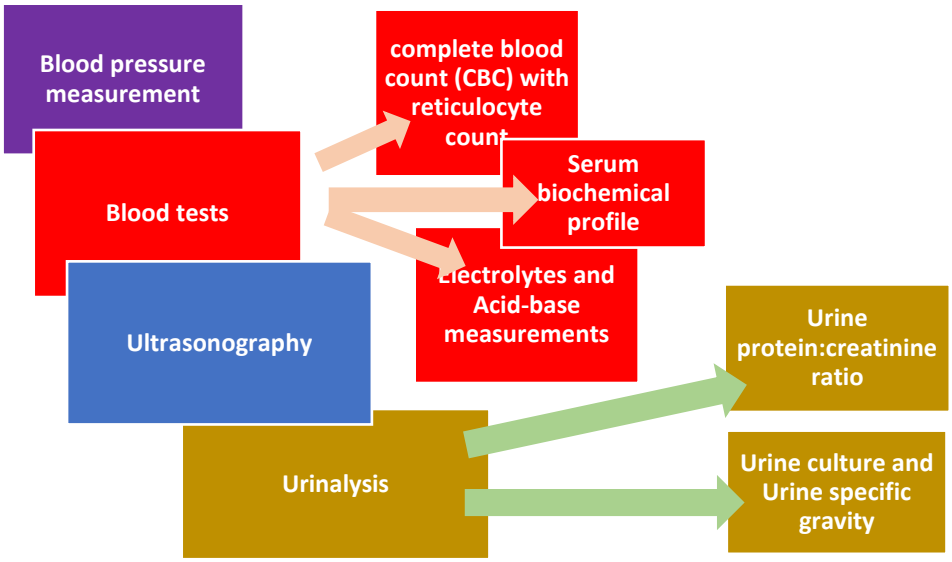

Fig. 2. Scheme of a basic diagnostic plan

Ultrasonography (US) findings (Fig. 3) were specific for chronic kidney disease (small kidneys, decreased cortico-medullary differentiation and mild hyperechoic cortical tissue). These findings are common in advanced stages of CKD and were considered indicative of an irreversible condition and poor prognosis (1).

Increased cortical echogenicity and decreased corticomedullary differentiation are reported as common signs of CKD in dogs (8).

Complete Blood Count and blood smear revealed mild leukocytosis: 27.6 $10^3/mm^3$, reference range (RR): 6-17 $10^3/mm^3$), mild thrombocytopenia: 190 $10^3/mm^3$ (RR: 200-500 $10^3/mm^3$), monocytosis: 2.6 $10^3/mm^3$, granulocytosis: 30.7 $10^3/mm^3$ (RR: 3-12 $10^3/mm^3$), moderate normocytic normochromic anemia (hematocrit 28% - RR: 37-55%, MCV, MCHC - within the reference range) with no evidence of regenerative response hemoglobin 10 g/dL - RR: 12-18g/dL, red blood cells 3.65M/µL - RR: 5.5-8.5M/µL. This type of anemia may be associated with chronic kidney disease. Considering the leukocytosis, an inflammatory process may be suspected as an underlying cause of kidney disease when related to the US findings.

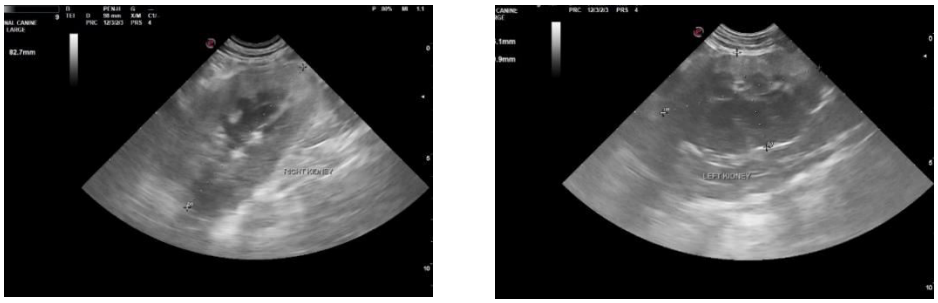

Fig. 3. Chronic renal disease with increased echogenicity and reduced cortical thickness in both kidneys

Blood biochemistry revealed severe azotemia: BUN: 183 mg/dl (RR: 1-29 mg/dl) and SCre: 10.8 mg/dl (RR: 0.2-1.4 mg/dl), mild elevation of liver enzymes: GGT: 24 IU/L (RR: 1-10 IU/L), GOT: 89 IU/L (RR: 10-50 IU/L), Total Bilirubin (T-Bil): 0,7 mg/dl (RR: 0.1-0.3 mg/dl), Total Cholesterol (T-Cho): 357 mg/dl (RR: 120-360 mg/dl), normal blood glucose level: 88 mg/dl (RR: 65-120 mg/dl), normal albumin: 2.6 g/dl (RR: 2.3-3.8 g/dl) and hyperphosphatemia (Phos: 10 mg/dL, RR: 2.5-6.8 mg/dl) These findings confirm the severe azotaemia and indicate mild hepatic disease. For definitive diagnosis of kidney disease further investigations are needed.

Venous blood gases and electrolytes (VBGE) (Fig. 4) showed metabolic acidosis with decreased ability to ventilate, which may be indicative of respiratory acidosis. The patient was given crystalloids and amino acids as well as bicarbonate replacement.

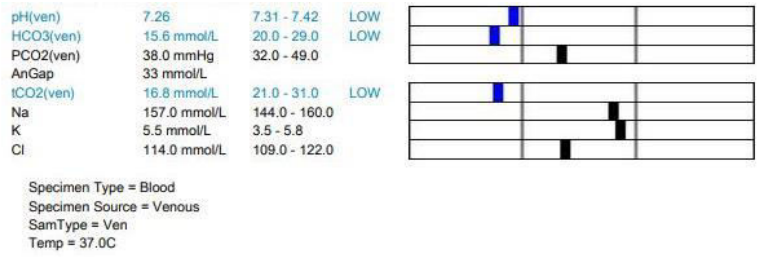

Fig. 4. Results of VGBE

Urinalysis: Urine Specific Gravity (USG): 1.020, pH 6.5, protein: 1000 mg/dL, while the rest of the parameters on the dipstick were negative. The sediment examination revealed an inactive sediment. The urine culture was negative. The USG value corelated with the azotaemia and dehydration indicates renal azotemia.

Because of massive proteinuria, further assessment is recommended, so Urine Protein/Creatinine (UP/C) ratio was performed. The degree of proteinuria yields useful information on the source of protein loss; namely losses of large amounts of protein (with high values of UP/C) is due to glomerular, and not tubular disease (14). Urine protein to creatinine ratios should only be performed on urine samples with evidence of excess protein (taking into consideration the USG) and the absence of lower urinary tract disease (6). In our case a UPC of 2.2, linked to the severe azotaemia, low USG and prehypertensive status suggest proteinuria of glomerular origin.

Considering all diagnostic findings, the dog was hospitalized, and supportive fluid therapy was provided. After 24 hours of constant rate infusion, it was considered that the patient was well hydrated, so another blood sample was collected to reassess kidney function and glomerular filtration rate. Because GFR measurements are laborious and expensive, surrogate plasma markers of reduced GFR like SDMA are used frequently. SDMA is the amino acid arginine that contains two methyl groups (dimethyl) in a symmetrical orientation and is considered a sensitive and early marker of declining GFR in dogs. Unfortunately, Diana's kidney parameters were even more elevated: SCre 13.5 mg/dl, BUN over 200 mg/dl and SDMA 1.18 µmol/L (RR under 68 µmol/L).

Diana was diagnosed with CKD-stage 4 according to current IRIS guidelines. Routinely, once diagnosed, kidney disease should be followed by **renal biopsy** to determinate the nature of injury, the underlying cause and to formulate a therapeutical plan according to this. It is advisable that this recommendation be adapted to each patient considering their clinical condition. A biopsy should be performed only when the results are likely to be valuable in the management of the case and when there are no contraindications (uncontrolled hypertension, coagulopathy or severe azotemia) (12, 15). In our case, due to persistent and severe azotemia despite continuous fluid therapy, we concluded that biopsy would not change the management of the case.

Follow-up: Diana died the next day, and their caregivers accepted a post-mortem renal biopsy to determine the underlying cause. The World Small Animal Veterinary Association – Renal Standardization Study Group (WSAVA - RSSG) recommend evaluating canine renal biopsies with LM (Light Microscopy), IF (Immunofluorescence) and TEM (Transmission Electron Microscopy) for a reasonable diagnostic workflow. Because veterinary electron microscopy is not available in our country, only LM and IF were performed. Results revealed glomerular and interstitial inflammation, fibrosis, tubular atrophy, and the definitive diagnosis was chronic immune-mediated glomerulonephritis.

Conclusions

First, for any disease, a detailed diagnostic plan is needed to detect which system is involved. In our case, the data suggested pathology of the excretory system. Further assessment of the kidney function was possible with the aid of several diagnostic tools. These may also identify underlying causes, ongoing renal injury, consequences of CKD and provide information about prognosis and treatment goals and necessity for further testing.

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RESEARCH IN PASTEURELLOSIS OF DOMESTIC RABBITS FROM EXTENSIVE GROWING

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Summary

The paper presents the results of investigations performed on a number of 154 rabbits, from 5 locations, females and males, of different ages and breeds. The rabbits showed the following symptoms: sneezing, whitish nasal and ocular secretions, subcutaneous abscesses in the region of the head, neck, and forelimbs. With a lower frequency, but still considerable, there were abscesses on the nipple chain in some lactating females, but also in a dry period. Otitis externa has also been reported in 2 rabbits, one of these also has torticollis. At the necropsy examination, lesions characteristic of pasteurellosis were highlighted, the diagnosis was confirmed by bacteriological examination. Antibiotic susceptibility testing was performed for the proper choice of antimicrobial substances for therapy.

Keywords: *Pasteurella multocida*, rabbits, pasteurellosis, antimicrobial susceptibility.

Respiratory infections are a major problem in rabbits, which cause considerable damage to farms and rabbit farms, which are represented by the loss of specimens, and the costs of prophylaxis and control. The main etiological agent incriminated is *Pasteurella multocida*, bacteria being most often associated with respiratory infections but can also cause subcutaneous pyogenic infections and localizations in various organs, or even sepsis, being one of the main causes of morbidity in rabbits (12, 14, 20).

Pasteurella multocida is a conditioned pathogenic microorganism that exists in the normal microflora of the mouth, nasopharynx, and the upper respiratory tract of birds, mammals, and other species (1, 2, 3, 24).

Pasteurella multocida infection produces many clinical signs in many species: atrophic rhinitis in pigs, cholera in birds in poultry, and hemorrhagic sepsis in buffalo and cattle (15, 16, 17, 19, 23).

Materials and methods

In the study, we examined over time the rabbit from 5 locations of the rabbitries, a total of 154 animals, females, and males, of varying ages and breeds. Epidemiological, clinical, necropsy examinations were performed, and the disease was confirmed by bacteriological examination.

Samples were collected from dead rabbits with respiratory diseases from the 5 locations. Each sample collected was placed in a collection tube and sent to the laboratory of bacterial infectious diseases, within FMV Timisoara.

The following breeds were present in the 5 locations: Checkered Giant rabbit, Rex rabbit, Panoniya rabbit, Miniature Papillon rabbit, and German Lop rabbit aged between 2 months and 3 years.

Brain Heart Infusion BHI and 5% ram's blood agar and cultured at 37°C for 24-48 hours were used for bacteriological examination. The identification and confirmation of bacterial species were by Gram staining and biochemical methods. On the agar-blood plates, after 48 hours of incubation, large, non-hemolytic, gray to gray-white mucoid colonies were observed The final identification of the isolates was made on the API 20NE kit (31).

Antibiotic sensitivity testing was performed by Kirby Bauer's diffusimetric disc method, using the following antimicrobial substances: amoxicillin (AML), ciprofloxacin (CIP), doxycycline (DO), enrofloxacin (ENR), florfenicol (FFC), gentamicin (GMN), neomycin (N), trimethoprim/sulfamethoxazole (SXT), tetracycline (TE) and penicillin (P).

The inhibition zone diameters were measured and rated as sensitive (S), intermediate (I), and resistant (R) according to the European Committee on Antimicrobial Susceptibility Testing (30).

Results and discussions

The rabbits showed the following symptoms: sneezing, whitish nasal and ocular secretions, subcutaneous abscesses in the region of the head, neck, and forelimbs. With a lower frequency, but still considerable, there were abscesses on the nipple chain in some lactating females, but also in the dry period. Otitis externa has also been reported in 3 rabbits, one of these also has torticollis (Table 1, Fig 1).

At the necropsy examination, lesions characteristic of pasteurellosis were highlighted, the diagnosis was confirmed by bacteriological examination on API 20NE.

The API 20NE kit correctly identified all the isolates from the affected rabbits, which have biochemical properties specific for the identification of Pasteurella multocida.

Table 1

Distribution of rabbits by location, lesions, and number of isolated *Pasteurella* strains

Location	**A**		**B**		**C**		**D**		**E**		**Total**
Total number of rabbits	42		49		31		16		16		154
Gender M/F	9M	33F	16M	33F	7M	24F	3M	13F	4M	12F	47M/ 107F
No of the affected rabbits	6	9	1	6	1	4	1	2	0	3	9M/ 24F
-Pneumonia	2	1		2	1	2					8
-Rhinitis	1	1		2		1		2		2	9
-Sinusitis	1										1
-Metritis		4									4
-Otitis	1	1	1								3
-Subcutaneous abscess	1	2		2		1	1			1	8
Number of *Pasteurella* isolates	**2**	**4**	**1**	**2**	**1**	**1**		**1**		**1**	**13**

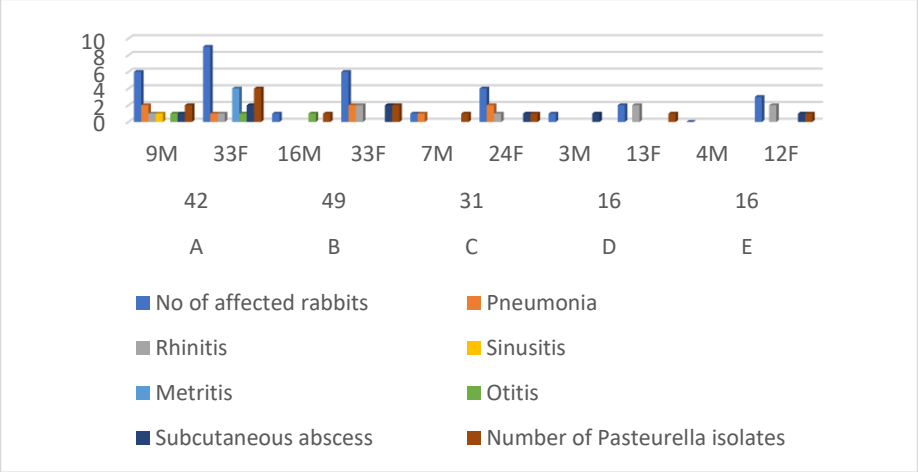

Fig. 1. Distribution of lesions on the locations

It was observed that in rabbits from farms where antibiotics were administered preventively, the phenomenon of multiple antibiotic resistance appeared (Table 2, Fig. 2).

Table 2

Synoptic of antibiotic susceptibility of 13 isolates of *Pasteurella multocida*

Antibiotics	Sensitive (S)		Intermediate (I)		Resistant (R)	
	No strains	(%)	No strains	(%)	No strains	(%)
Gentamicin (GMN) 10 µg	7	53.9	3	23.1	3	23.1
Trimethoprim/sulfamethoxazole (SXT) 30 µg	6	46.2	4	30.8	3	23.1
Enrofloxacin (ENR) 5 µg	7	53.8	2	15.4	4	30.8
Neomycin (N) 10 µg	2	15.4	6	46.2	5	38.5
Amoxicillin (AML) 10 µg	3	23.1	2	15.4	8	61.5
Ciprofloxacin (CIP) 30 µg	8	61.5	3	23.1	2	15.4
Doxycycline (DO) 10 µg	3	23.1	1	7.7	9	69.2
Tetracycline (TE) 30 µg	3	23.1	0	0.0	10	76.9
Penicillin (P) 10 µg	13	100.0	0	0.0	0	0.0
Florfenicol (FFC) 10 µg	9	69.2	2	15.4	1	7.7

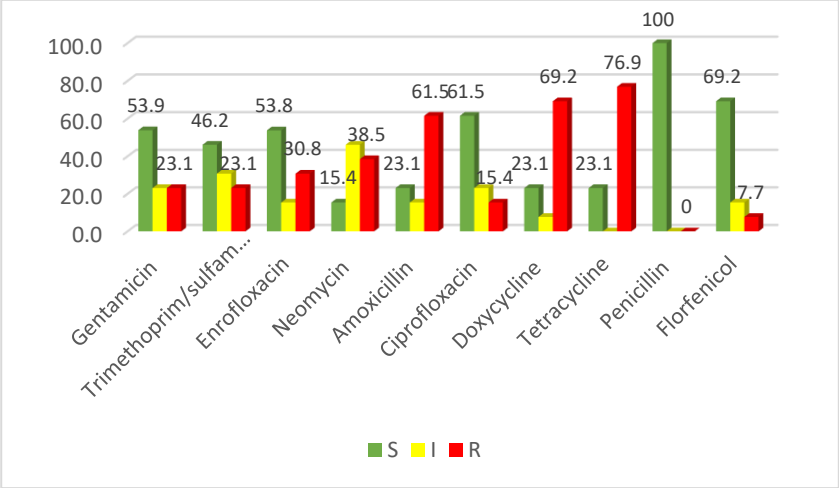

Fig. 2. Dynamics of antibiotic susceptibility of 13 *Pasteurella multocida strains*

The 13 isolated *Pasteurella multocida* strains were sensitive (100%) to penicillin, florfenicol (69.2%), ciprofloxacin (61.5%), gentamicin (53.9%), and enrofloxacin (53.8%).

The highest resistance of *Pasteurella multocida* strains was found in tetracyclines (76.9%), doxycycline (69.2%), amoxicillin (61.5%), antibiotics commonly used in the treatment of rabbits.

In a report of the incidence of pasteurellosis on a rabbit farm, the clinical signs observed in this report, namely. Nasal secretions, pyrexia, sneezing, conjunctivitis, dyspnea, and abdominal breathing have also been reported (26).

Reported post-mortem and histopathological results, such as hemorrhagic pneumonia, hepatic and splenic congestion, tracheitis, etc. The culture antibody showed a higher sensitivity to ceftiofur followed by enrofloxacin, gentamicin, chloramphenicol, and tetracycline and was resistant to ampicillin, neomycin, and azithromycin (21, 22).

Other authors found that *P. multocida* type B isolates from pigs and cattle with acute sepsis had a 98% susceptibility to ceftiofur followed by 86% to enrofloxacin, 84% to gentamicin, 81% to chloramphenicol (6).

A study conducted on samples collected from 50 dead rabbits, revealed 23 suspicious strains from 50 lung samples, representing 46%, from the liver, were isolated 11 strains (22%), from the heart 13. The biochemical examination showed that 11 out of 23 suspected *Pasteurella* isolated from lung tissue had a typical *Pasteurella multocida*, 3 out of 11 suspected *Pasteurella* isolated from liver tissue had a typical *Pasteurella multocida* and 6 out of 13 isolated suspected *Pasteurella* were confirmed as typical *Pasteurella multocida* (7, 8, 10, 11, 27).

A report in Egypt, where all strains of rabbit *Pasteurella multocida* were examined, showed remarkable MDR patterns (9, 18, 21).

The presence of isolated multidrug-resistant *Pasteurella multocida* recovered from rabbits affected by pasteurellosis has already been reported in Italy (5).

In another study in Brazil, (47.8%) (22/46) of *Pasteurella multocida* rabbit strains were resistant to at least one of the drugs tested (13).

Antibiotic resistance is a growing problem, probably attributed to the extensive and indiscriminate use of antibiotics to prevent and treat pasteurellosis.

Antibiotic susceptibility testing of isolated strains showed that they were multidrug-resistant (MDR) with a predominant resistance to erythromycin and oxytetracycline (100%), followed by kanamycin and ceftriaxone (80%). They were sensitive to trimethoprim/sulfamethoxazole, gentamicin, amoxicillin, amikacin, ampicillin, and chloramphenicol (25, 29).

Rabbits can be potential sources of *Pasteurella multocida*, multidrug-resistant with subsequent negative implications significant effects on public health.

Conclusions

Pasteurellosis was diagnosed in rabbits of different ages and breeds at the locations studied.

Pasteurella multocida was the main etiological agent of respiratory infections in rabbits, but also produced pyogenic infections with multiple localizations in the body.

Pasteurella multocida strains isolated from those outbreaks showed the phenomenon of antibiotic resistance to tetracyclines (76.9%), doxycycline (69.2%), amoxicillin (61.5%).

Pasteurella multocida strains were sensitive (100%) to penicillin, florfenicol (69.2%), ciprofloxacin (61.5%), gentamicin (53.9%), and enrofloxacin (53.8%).

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GENTIANA ASCLEPIADEA: *IN VITRO* EVALUATION OF OVICIDAL AND LARVICIDAL EFFECTS

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Summary

The research was conducted at the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca between October 2020 and October 2021. The aim of the study was to determine *in vitro* the possible ovicidal and larvicidal effects of 70% ethanol extract of *Gentiana asclepiadea* roots on donkey strongyle. The anthelmintic potency of the extract was evaluated using egg-hatching assay (EHA) and larval development assay (LDA). The objectives of the research were to test the potential *in vitro* anthelmintic activity of *G. asclepiadea* alcoholic extract and to quantify the biologically active compounds of plant extract. This plant was chosen because it contains bitter substances (e.g. gentiopicrozide, oligosaccharides gentianose, phenolic acids, tannins) that stimulate the entire digestive system (salivary secretions, the liver, glands with internal secretion). It is also known that standardized preparations based on gentian have antipyretic, antimalarial, antibacterial, and anthelmintic action. Fecal samples from fifteen donkeys raised in a family farm located in Cluj County were collected for the testing. The obtained egg suspension was used for the following determinations: egg-hatching assay (EHA), larval development assay (LDA), and larval identification (preparation of faecal culture). The obtained results were analysed statistically and the mean reduction (%) of egg hatch and larval development were calculated. The total phenolic content of plant extract was determined using the Folin-Ciocalteu method, and total flavonoid concentration using the colorimetric assay kit (Elabscience Biotechnology Inc.). The study showed that the tested plant extract express strong ovicidal and larvicidal *in vitro* activity against donkey gastrointestinal nematodes at a concentration of total phenolic content higher than 75 µg/mL.

Keywords: plant extract, *Gentiana asclepiadea*, anthelmintic, EHA, LDA.

Antimicrobial and anthelmintic resistance has become a serious threat to public health around the world (16). The rapid evolution and expansion of this phenomenon implies potential consequences for both domestic animals and humans. The study of medicinal plants and their secondary metabolites might provide some extracts with novel structures or different mechanisms of action, which in turn would make good substitutes for the currently used drugs that are raising concern.

Currently, the knowledge in veterinary phytotherapy is based on a small number of veterinary phytotherapy books, few *in vitro* and *in vivo* studies and mainly on the adaptation of human phytotherapy and existing empirical evidence. It is

certain that the study of the bioactive potential of plants and their secondary metabolites is still in its early stages. Out of the 250,000 - 500,000 existing plant species, only a tiny proportion were studied in order to scientifically demonstrate their therapeutic effect and analysed in terms of composition (14, 19).

Grazing farm animals are naturally exposed to gastrointestinal (GI) nematodes. These infections are often associated with subclinical disease resulting in reduced growth rate, poor body condition, abdominal distension, poor reproductive performance, caused by poor feed conversion rate, resulting from appetite suppression, diarrhea and colic (1, 15). The importance of proper prophylaxis against these parasitosis also lies in the fact that the affected animals have production loses, among other clinical signs (13). Due to donkey's milk biochemical composition, it is considered to be the most similar to breast milk, being used in infant nutrition and by people with lactose intolerance (2). Moreover, studies conducted by Derdak et al. (7) proved it has multiple properties including antioxidant, anti-inflammatory, antimicrobial, antiviral and antiproliferative activities. All these benefits made donkey's milk to be use in food, pharmaceutical and cosmetics industries (3, 8).

Although the available information on how to administer, dose, side effects and waiting time for herbal preparations is currently limited for dehelmintization, some farmers use wormwood (*Artemisia absinthium*), wormwood (*A. vulgaris*), common chicory (*Cichorium intybus*) and vetch *(Tanacetum vulgare*) for both treatment and control of GI nematodes. Alternative prophylaxis and curative methods are needed due to the fact that the incorrect usage of molecules like bezimidazoles, tetrahydroxypyrimidines and macrocyclic lactones created resistance against gastrointestinal nematodes in donkeys (2, 10).

Even though there has been numerous scientific studies which have succeeded in demonstrating the *in vitro* efficacy of plant extracts, *in vivo* testing is limited and claims on the efficacy of plant-based products cannot be validated because the experiments cannot be reproduced. An important cause is the lack of information on the composition of the plant extracts, the method of preparation and the administered dose. In addition, significant differences in the concentration of active compounds of plant extracts, caused by genetics, soil, temperature, humidity and harvest period, can lead to changes in the therapeutic properties of plant-based products (20).

The aim of our study was to assess the possible ovicidal and larvicidal effect of *Gentiana asclepiadea* root extracts on donkey strongyle.

Materials and methods

Preparation of plant extracts

Gentiana asclepiadea (willow gentian) is often found in the composition of anthelmintic tea mixtures according to its ethno-therapeutic use. Plant materials were purchased in September 2020, from a local licensed herbal store.

The 70% ethanol plant extract was prepared by adding 10g of powdered rhizomes and roots to 100 mL of 70% ethanol. The suspension was mixed for 30 minutes, stored in a dark place, at room temperature, for 14 days, being mixed for 5 minutes each 12 hours. After 14 days the obtained extract was centrifuged (4000 RPM, 10 minutes) and filtered through Whatman qualitative filter paper no.1 (11 µ particle retention). The final volume of plant extract was 78 mL. It was stored at 4°C until further use.

Egg recovery and preparation

In this study, fifteen donkeys from a family located in Cluj County were included. The females were of 3-5 years, clinically healthy and not treated with anthelmintics for at least 6 months before the study (being considered naturally infected with gastro-intestinal parasites). Fecal samples were collected directly from rectum using sterilised gloves and collecting bags.

The samples were mixed with warm distilled water and filtered through 250, 125 and 25 µm sieves. The 25 µm sieve is supposed to retain the strongyle eggs (the eggs being bigger). The eggs were washed off the sieve with distilled water and it was collected in sterile tubes. The tubes were centrifuged (4000 RPM for 5 minutes) and the supernatant was removed. The sediment which contained eggs was collected and analysed by the McMaster counting method. The sediment was diluted with distilled water to obtain a suspension with 300 eggs in 100 µL.

Egg hatching assay (EHA)

The EHA was performed after the method described by Coles et al. (5), with some modifications. 24-well cell culture plates were used, in each testing well adding 800 µL of distilled water, 200 µL of egg suspension and 1000 µL of 70% ethanol plant extract. The final dilutions of aqueous plant extract are listed in Table 1. For each concentration, four replicates were tested, together with two negative controls for each concentration (total of 12 negative controls). For these wells, willow gentian extract was replaced by 1000 µL of 70% ethanol. The plates were incubated at 27°C for 48h.

The number of eggs and first-stage larvae (L1) were counted using an optical microscope. The following formula was used to calculate the egg hatching percentage:

$$\% \text{ hatched eggs} = \frac{L1}{L1 + \text{eggs}} \times 100$$

Larval development assay (LDA)

For the determination of the potential larvicidal effect of the aqueous plant extract, larval development assay was used. LDA was performed using the same six concentrations of 70% ethanol willow gentian extract, as for the EHA (2.5, 10.0, 50.0, 75.0, 150.0 and 1000 µg/mL), having four replicates and two negative controls for each dilution. The mix of 800 µL of distilled water and 200 µL of egg suspension was incubated at 27°C for 48h. After two days, 1000 µL of plant extract were added to each well and let in the incubator for 14 more days at 2°C in order to obtain third

stage larvae (L3) (6, 18). For the wells with negative control, the aqueous plant extract was replaced with the same volume of distilled water, the same protocol being used. The larval development percentage was calculated using the following formula:

$$\% \text{ larval development} = \frac{L3}{\text{unembryonated eggs} + L3} \times 100$$

Table 1

Concentration and volume of plant extracts added to wells

Concentration (µg/ml)	Volume of extract (µl)	Volume of distilled water (µl)
2.5	2.34	997.66
	1.99	998.01
10.0	9.36	990.64
	7.96	992.04
50.0	46.8	953.2
	39.82	960.18
75.0	70.0	930.0
	59.73	940.27
150.0	140.4	859.6
	119.46	880.54
1000.0	936.0	64.0
	796.0	204.0

Larval identification

For the larval identification, faecal culture was prepared from the mixed sample of collected faeces. In a plastic cup, a small quantity of faeces was mixed with distilled water and after that was covered with perforated aluminium foil. The sample were incubated for 14 days at a temperature of 27°C, time necessary for development of third stage larvae (L3). After 14 days the L3 were collected and examined using optical microscopy. The morphological identification of larvae (200 L3) was performed based on the length of intestine, number of intestinal cells, shape of the cells and their arrangement (4, 17). Based on that the strongyles were classified into subfamilies *Cyathostominae* and *Strongylinae*.

Statistical evaluation

The results from EHA and LDA were analyzed using excel and the mean reduction (%) of egg hatch and larval development was calculated for each dilution and negative control.

Phytochemical investigations

Total phenolic content

Polyphenols in plant extracts react with specific redox reagents to form a blue complex that can be quantified by visible-light spectrophotometry. For the determination of total phenolic content, the Folin-Ciocalteu assay was used.

For the calculation of the standard curve as a reference was used galic acid. From the galic acid stock solution of 1 mg/ml, through serial dilutions, solutions with following concentrations were prepared: 100, 50, 25, 12.5, 6.25 and 3.175 µg/ml. For each concentration, 1 ml of solution was mixed with 5 ml of Folin-Ciocalteu reagent (reagent A). After thorough mixing, 4 ml of alkaline working solution (reagent B) were added. Prepared standard solutions were then let to rest for 90 minutes at the room temperature. After 90 minutes, the absorbance was measured at 765 nm, and the standard curve was calculated.

For the plant extracts the same steps were followed, galic acid dilutions being replaced with 1 ml of extract. Determination of total phenolic content was performed in correlation with standard curve.

Total flavonoids

Total flavonoids were quantified using Plant Flavonoids Colorimetric Assay Kit (Elabscience Biotechnology Inc.). In alkaline nitrite solution, flavonoids form red complex with aluminium ion. The flavonoid content in the sample is then calculated by measuring the absorptivity of the sample extract at 510 nm using a spectrophotometer. 540 µl of plant extract was added to the tube and mixed with 30 µl of Reagent 2. Obtained solution was homogenised and let to stand for 5 minutes at room temperature. After that 30 µl of Reagent 3 was added to the sample, followed by homogenisation and resting for 5 minutes at room temperature. Next, 40µl of Reagent 4 were added to the sample, mixed and let to stand at room temperature for 15 minutes. In the standard tube the extract was replaced with different concentrations of standard solution, in the blank tube – with distilled water. Flavonoid concentration (mg/g) was then calculated using the formula given in the instructions attached to the kit.

Results and discussions

Egg hatching assay and larval development assay

The results of EHA for *G. asclepiadea* found in Table 2 indicate that only 3 out of 6 concentrations have a significant ovicidal effect. *G. asclepiadea* starts to have a good ovicidal effect at a concentration of 75 µg/mL with an inhibition of 77.14% and at a concentration of 1000 µg/mL there is a 100% inhibition thus as the

concentration increases so does the inhibition. The results of egg hatching assay with a 70% ethanol control can be found in Table 3.

Table 2

Egg hatching assay results – *Gentiana asclepiadea*

Sample number	Concentration (µg/ml)	Eggs	Larva	Total eggs	Total larva	% inhibition	mean % inhibition
1.1.	2.5	4	36	33	173	11.475	16.02
1.2.		5	23			11.86	
1.3.		6	56			10.91	
1.4.		18	58			19.75	
2.1.	10	27	27	62	147	36.11	29.67
2.2.		18	35			42.42	
2.3.		14	36			44.62	
2.4.		3	49			45	
3.1.	50	35	11	98	92	63.77	51.58
3.2.		22	22			75	
3.3.		18	32			84.21	
3.4.		23	27			88.88	
4.1.	75	29	9	108	32	94.23	77.14
4.2.		29	10			52.72	
4.3.		36	10			97.22	
4.4.		14	3			96.77	
5.1.	150	28	18	155	35	82.98	81.58
5.2.		42	0			91.66	
5.3.		56	7			77.14	
5.4.		29	10			84	
6.1.	1000	63	0	204	0	100	100.00
6.2.		46	0			100	
6.3.		39				100	
6.4.		56				100	

Table 3

Egg hatching assay results – 70% ethanol control for *Gentiana asclepiadea*

Control number	Volume (µL)	Eggs	Larva	Total egg	Total larva	inhibition %	Total inhibition %
1.1.	1.99	6	45	14	92	11.76	13.21
1.2.		8	47			14.54	
2.1.	7.96	10	50	14	106	16.67	11.67
2.2.		4	56			6.67	
3.1.	39.82	3	38	11	90	7.32	10.89
3.2.		8	52			13.33	
4.1.	59.73	4	57	10	111	6.56	8.26
4.2.		6	54			10	
5.1.	119.46	7	33	10	76	17.5	11.63
5.2.		3	43			6.52	
6.1.	796	33	30	82	56	52.38	59.42
6.2.		49	26			65.33	

The results of larval development assay can be found in Table 4. *G. asclepiadea* only starts to have a significant larvicidal effect at a concentration of 75 µg/mL with an inhibition of 95.24%. From the obtained results, can be concluded that only 3 out of 6 concentrations have significant larvicidal effect. The larval development assay results with 70% ethanol control can be found in Table 5.

Table 4

Larval development assay results – *Gentiana asclepiadea*

Sample number	Concentration (µg/mL)	Eggs, L1, L2	L3	Total eggs, L1, L2	Total L3	% inhibition	mean % inhibition
1.1.	2.5	2	14	27	114	12.5	19.15
1.2.		11	32			25.58	
1.3.		9	40			18.37	
1.4.		5	28			15.15	
2.1.	10	23	40	97	155	36.51	38.49
2.2.		29	47			38.16	
2.3.		24	36			40	
2.4.		21	32			39.62	
3.1.	50	22	8	72	38	73.33	65.45
3.2.		12	8			60	
3.3.		14	9			60.87	
3.4.		24	13			64.86	
4.1.	75	16	1	80	4	94.12	95.24
4.2.		22	1			95.65	
4.3.		24	1			96	
4.4.		18	1			94.74	
5.1.	150	14	1	85	2	93.33	97.70
5.2.		23	0			100	
5.3.		26	0			100	
5.4.		22	1			95.65	
6.1.	1000	12	0	81	0	100	100.00
6.2.		10	0			100	
6.3.		28	0			100	
6.4.		31	0			100	

Fig. 1 indicates that as the concentration of willow gentian increases it has a far greater inhibition than that of the control that was used.

Fig. 2 shows that willow gentian ethanolic root extract has a higher inhibition at all concentrations when compared to the ethanolic control.

Table 5

Larval development assay results – 70% ethanol control for *Gentiana asclepiadea*

Control number	Volume (µL)	Eggs, L1, L2	L3	Total eggs, L1, L2	Total L3	inhibition %	Total inhibition %
1.1.	1.99	2	15	6	40	11.76	13.04
1.2.		4	25			13.79	
2.1.	7.96	3	30	4	48	9.09	7.69
2.2.		1	18			5.26	
3.1.	39.82	6	34	10	50	15	16.67
3.2.		4	16			20	
4.1.	59.73	3	13	6	28	18.75	17.65
4.2.		3	15			16.67	
5.1.	119.46	8	27	10	36	22.86	21.74
5.2.		2	9			18.18	
6.1.	796	7	14	13	25	33.33	34.21
6.2.		6	11			35.29	

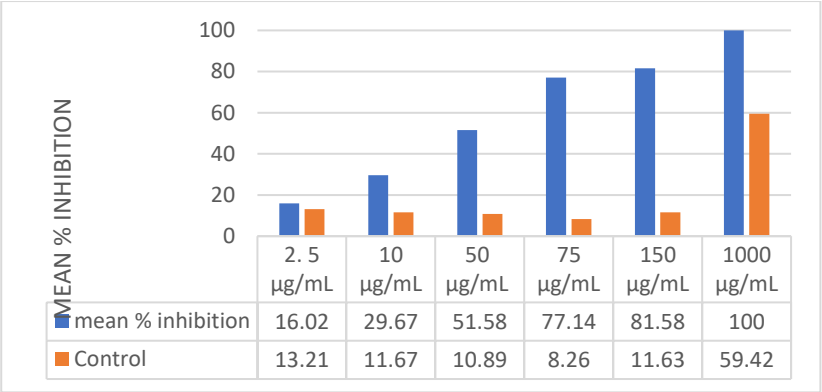

Fig. 1. EHA mean inhibitory % of *Gentiana asclepiadea* vs control

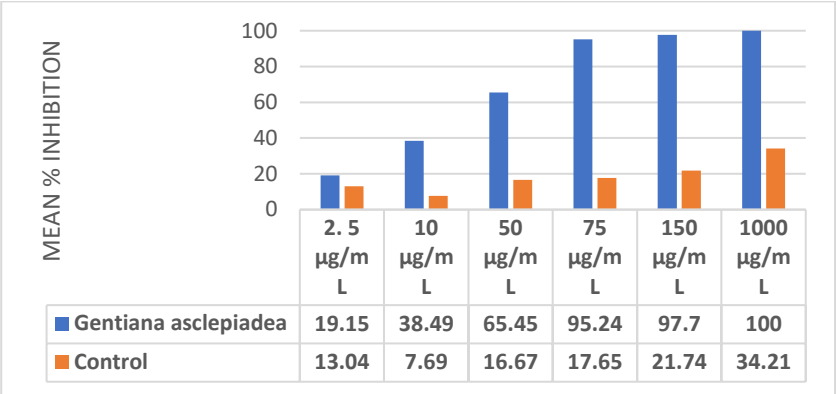

Fig. 2. LDA mean inhibitory % of *Gentiana asclepiadea* vs control

Fig. 3 compares willow gentian ovicidal and larvicidal abilities. From the concentration of 2.5 µg/mL to 150 µg/mL *G. asclepiadea* has a higher larvicidal effect. At the concentration of 1000 µg/mL both EHA and LDA have 100% inhibition.

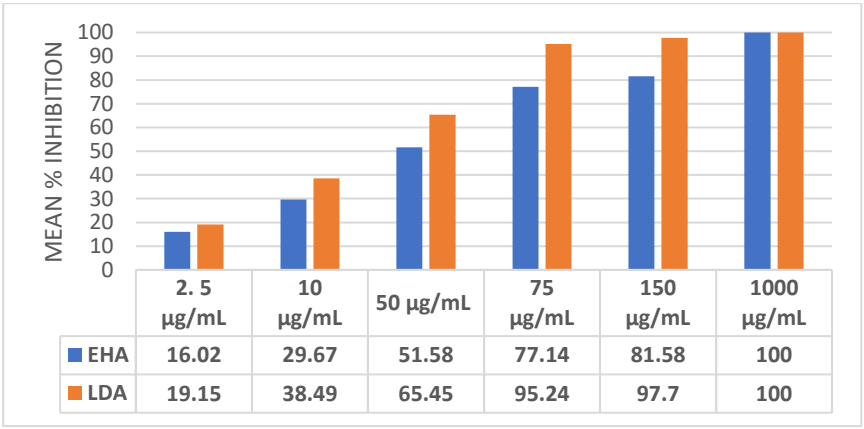

Fig. 3. EHA vs LDA mean inhibitory % of *Gentiana asclepiadea*

Experiments showed that willow gentian roots extract has antioxidant effects and antigenotoxic effect against DNA damaged induced by alkylation with ethyl methanesulfonate (EMS) in *Drosophila melanogaster* (12). *G. asclepiadea* is also found in tea mixtures used by humans for treating gastrointestinal problems and parasitic infections (2). There are also studies which show that gentian extracts have efficiency against donkey strongyles (2).

Larval identification

The results of larval identification for subfamily *Cyathostominae, Strongylinae* and *Trichostrongylinae* are presented in the Fig. 4 - 6.

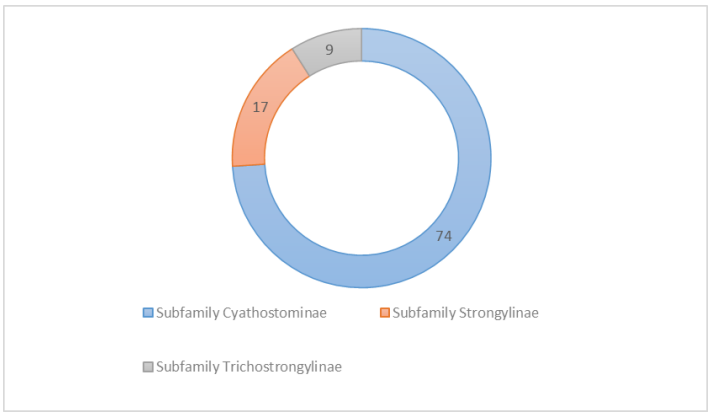

Fig. 4. Total percentage of identified strongyle subfamilies

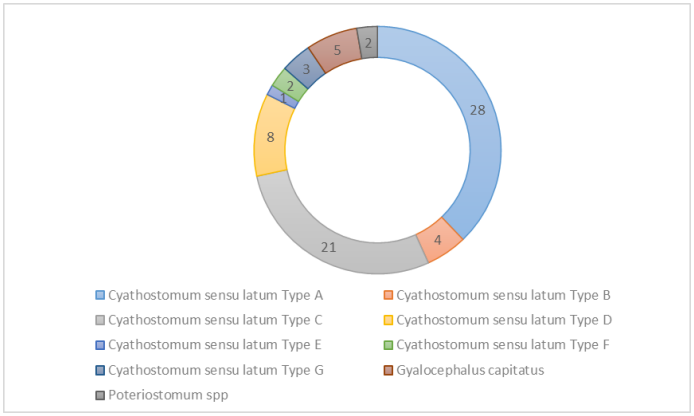

Fig. 5. Identified species of *Cyathostominae* subfamily

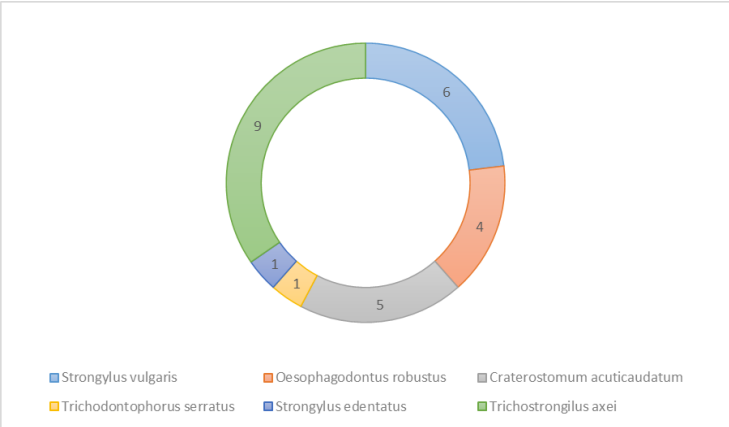

Fig. 6. Identified species of *Strongylinae* and *Trichostrongylinae* subfamilies

From the total number of examined L3 larva 74% belong to the subfamily *Cyathostominae*, the most abundant one being *Cyathostomum sensu latum* type A (28%). Followed by subfamilies *Strongylinae* and *Trichostrongylinae*, with 17% and 9% respectively. The most frequently identified species of *Strongylinae* subfamily being *Strongylus vulgaris* with 6%.

Similar prevalence of donkey strongyles was found by Matthee et al. (9) and Matthews et al. (11) in their studies.

Phytochemical investigation

Following the analysis of the results obtained, can be observed that the quantity of polyphenols in the 70% ethanolic extract of *Gentiana asclepiadea* root is 19.578 mg/g plant material and the content of total flavonoids is 2.033 mg/g plant material. Total phenolic and flavonoid content (mg) was also calculated for 1 mL of extract (Table 6).

Table 6

Concentration of total phenolic and flavonoid content

Total phenolic concentration		Total flavonoid concentration	
mg/g plant material	mg/ml extract	mg/g plant material	µg/ml extract
19.578	2.511	2.033	260.75

Conclusions

Willow gentian showed strong ovicidal effect at 75 µg/ml and higher concentrations with a mean reduction percentage of 77.14%.

G. asclepiadea indicated significant larvicidal effect at the concentration of 75 µg/ml and a mean reduction percentage of 95.24%.

74% from the total number of examined L3 larva belong to the subfamily *Cyathostominae*, the most abundant one being *Cyathostomum sensu latum* type A (28%).

Phytotherapy shows potential in being used as a good alternative to products that are currently on the market and would benefit from further research.

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CLINICAL AND ANATOMOPATHOLOGICAL ASPECTS FOUND IN CALVES WITH RESPIRATORY DISEASES

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Summary

Respiratory diseases are common in cattle, especially in intensive breeding systems. In the present study, several calves from a fattening farm in Western Romania were examined clinically and anatomopathologically. They had respiratory symptoms and underwent various tests to determine the main biotic agents involved. The results of all investigations suggested a mixed infection with Bovine Parainfluenza Virus type 3 (BPIV3) and *Pasteurella multocida*. The source of infection was represented by the newly introduced calves on the farm and the observed results indicate major deficiencies and management errors in terms of isolation and early vaccination of animals.

Keywords: BRD, ruminants, BPIV3.

Bovine respiratory disease (BRD) in calves is a multifactorial syndrome characterized by acute, subacute or chronic inflammation of the lung airways and parenchyma (11, 17), age starting point of diagnostic being 3 weeks (24).

BRD have an increased impact on intensive cattle breeding and is also the leading cause of economic losses for young cattle (11), due to the weight loss and the costs of the treatments. It has an enzootic character. Over 10% of calves that are affected in fatteners have respiratory diseases and in these units up to 80% of existing calves can get sick (11, 24). The etiology of these infections involves abiotic and biotic factors, which are interconnected (11, 17).

Abiotic factors represented by stress, overcrowding, poor hygiene and nutrition conditions, deviations from growth technology, cause a decrease in general resistance and the appearance of micro-lesions of the respiratory mucosa, which are gateways for biotic factors (11, 25, 26, 30, 33).

There are several morphological features of cows, which can favor the appearance of BRD. A first aspect is that cattle do not have collaterals between bronchioles (an obstruction will affect the entire lung parenchyma served downstream), any rapid contraction of smooth muscles will increase blood pressure in vessels and therefore, the risk of pulmonary edema.

Another aspect is that the bovine lung is compact, well developed, which makes the breathing require a high energy consumption. Normally, the calves under

the age of 12 months, have not yet reached the maximum of pO2 in blood, hence the absence of reserves in case of lung problems. The meat breeds have almost zero respiratory reserve, therefore in case of hypoxia, the hyperventilation that the body can produce has limited effectiveness. Also, the heart cannot cope effectively in case of tachycardia, the volume of the ejected blood being reduced and an increased pressure in the cranial vena cava can be produced (26).

The biotic factors are represented by viruses, bacteria and parasites that act directly on the respiratory system, or complicate the initial lesions caused by abiotic factors, producing respiratory infections with variable clinical and anatomical expression (11). A multitude of bacteria can be identified in the lungs: *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, *Arcanobacterium pyogenes*, *Streptococcus pneumonia*, *Staphylococcus aureus*, *Chlamydiales* spp., *Fusobacterium necrophorum*, *Corynebacterium bovis*, *Streptococcus* spp., *Micrococcus* spp. and *Mycoplasma* (24).

The viruses have a major role (11). The main viral agents responsible for the appearance of BRD are Bovine Syncytial Virus (BRSV) and Bovine Parainfluenza Virus (BPI3). In most cases, the infections are mixed with both viruses, which are part of the *Paramyxoviridae* family. BRSV is included in *Orthopneumovirus* genus and BPIV3 is currently called Bovine respirovirus 3 and is part of the genus *Respirovirus* (2, 31).

The viral disease is transmitted from one calf to another horizontally, by airway (25). An important factor influencing the maintenance and the recurrence of infections is represented by the subclinical evolutions (12). The infections with BRSV can be diagnosed throughout a calendar year, regardless of the season (5).

The study was carried out in order to obtain epidemiological and anatomoclinical data from a calve fattening farm, in western part of Romania.

Materials and methods

Farm details

The research was carried out between 2020 and 2021 in a calve fattening farm from western Romania, where respiratory infections were followed by epidemiological, clinical and pathological examinations.

The farm has a total population about 5.200 heads, aging from 2 weeks to around 2 years. The calves come from Romania (Timiș, Satu Mare and Bihor counties), Lithuania and Hungary.

There are two major sectors in the farm: the accommodation sector (lines 40, 30, 50) and the quarantine boxes. The line 40 includes 3 stables with 50 boxes, each stable with 9-10 calves per box, weighing over 400-500 kg. The line 30 includes 6 stables (16 boxes for each), with 10-15 calves per box and the line 50 contains 6 stables (20 boxes for each), with 20 calves per box. Lines 40 and 30 have wooden floors and artificial ventilation system. Line 50 has cemented floors and mechanical curtains set up to open automatically at a certain temperature.

Epidemiological examination

In order to establish a complete epidemiological analysis, the following aspects were investigated: the presence of respiratory infections, the sources through which the causative agents entered the farm, the affected age groups, the morbidity and the mortality.

Clinical examination

Clinical examinations were made up by age group, following the presence of respiratory symptoms corresponding to several diseases with clinical manifestations of this kind.

The animals were thermometered and a pulmonary auscultation was performed.

Pathological examination

Pathological examinations were performed on 30 corpses from animals that had shown respiratory signs prior to death. From these, the tracheobronchial tree, the mediastinal lungs and the lymph nodes were examined.

The histopathological examination was performed from the above mentioned organs, by classical methodology. The samples were fixed in absolute alcohol, included in paraffin, sectioned and stained by the HEA method. The histopathological examination was performed in the Laboratory of Anatomical Pathology (Faculty of Veterinary Medicine, Timișoara).

The aim of the research was to identify disease-specific lesions with clinical respiratory manifestations. The bacteriological and virological laboratory examinations were performed at the DSVSA Timiș laboratory. Lung fragments were taken and processed by mRT-qPCR method to detect BRSV, BoHV-1 and BPIV3. The protocol and the primers used were similar to those described by Thonur et al. (2012) (34).

Results and discussions

Epidemiological examination results

Epidemiological examinations confirmed that BRD was introduced in farm by the newly acquired calves, although at the entrance to the farm they were vaccinated and treated preventively according to the following scheme:

- vaccine against IBR (Bovine Infectious Rhinotracheitis), BPIV3 (Bovine Parainfluenza 3), BRSV (Bovine Syncytial Respiratory Virus), BVD (Bovine Virotic Diarrhea);
- treatment with antibiotics represented by tulatromycin and tildipirosin 40 mg administered i.m. and with tylosin and doxycycline in milk associated with a vitamin complex.

The use of tulatromycin or tilmicosin is recommended by researchers due to the high concentrations remaining in the body and their prolonged action (3). Other authors indicate the use of a nitric oxide releasing solution (NORS) in BRD prevention in calves due to its beneficial effect in increasing the immune response

(20, 32). In general, the preventive antibiotic therapy is applied to all newly acquired calves, this determining the appearance of antibiotic resistance, over time (4, 6, 7, 18, 19, 21).

A recent study looked at the use of a Whisper On Arrival system, which consists of applying a device on animal's body capable of monitoring several values for each calf (heart sounds, lung sounds, body temperature and body weight). Through the software contained, the data provided are analyzed and a clear answer is developed for each animal, whether or not it should be subjected to treatment. Basically, this system decides whether an animal is sick or not and whether it is necessary to apply an antibiotic therapy (27).

In veterinary practice, the vaccination of calves begins at the age of 2 weeks. An adequate immune response and protection occurs in 30-45 days. Thus, it is recommended to observe these considerations.

Clinical examination results

The laboratory results and the lessions observed in the organs confirmed BPIV3 and *Pasteurella multocida* infections.

Associations of BPI3 with BCV (Bovine Respiratory Coronavirus) have also been reported globally (29).

BPIV3 infection

The clinical examination was performed by age group throughout the study period. In sick calves, 40-41°C fever, serous discharge, dyspnoea and severe vesicular murmur were found. After a few days, cough, tracheal murmur and wet rales were observed. Calves that did not have tracheal complications healed after a week. Similar studies have reported persistent, dry cough and even mucoid or purulent oculonasal discharge (rhinitis being the cause) (14, 22).

Other research has shown the installation of pyrexia in calves, after 2 days from contact with the viral agent, the highest values of body temperature being observed at 4-5 days (1, 28). Superacute forms with a lethal end in 2-3 days have also been reported.

The immunity persistence following infection is not fully known, with situations of reinfestation in calves being encountered a few weeks after the initial exposure. The virus stays in the animal's body for months, so the risk of transmission to other animals is high (14, 35).

***Pasteurella multocida* secondary infection**

There were cases in which secondary bacterial infections overlapped, which manifested themselves clinically through: mucopurulent discharge, cough, severe dyspnea. The noises heard were characteristic for bronchopneumonia.

Significant weight loss has occurred, some of the calves have died after a few weeks, and those who have recovered have been left with lung sequelae.

Through the endotoxins they contain (leukotoxins and lipopolysaccharides), they act on the local defense system (lethal effect on alveolar macrophages) and the lung tissue, producing a degeneration of pneumocytes and capillary endothelium, followed by inflammatory cells migration and massive neutrophil exsudation. Lytic

enzymes and oxygen free radicals are released, producing multiple focal necrosis. Alteration of the vascular endothelium, accompanied by thrombosis, results in multiple foci of coagulative necrosis delimited by neutrophils and macrophages (23).

Pathological examination results

Macroscopic and microscopic lesions in BPIV3 infection

At necropsy in uncomplicated forms was found tracheitis, bronchitis, atelectasis in the cranial lobes, accompanied by pulmonary edema (Fig. 1 A), subpleural and interstitial emphysema (Fig. 1 B).

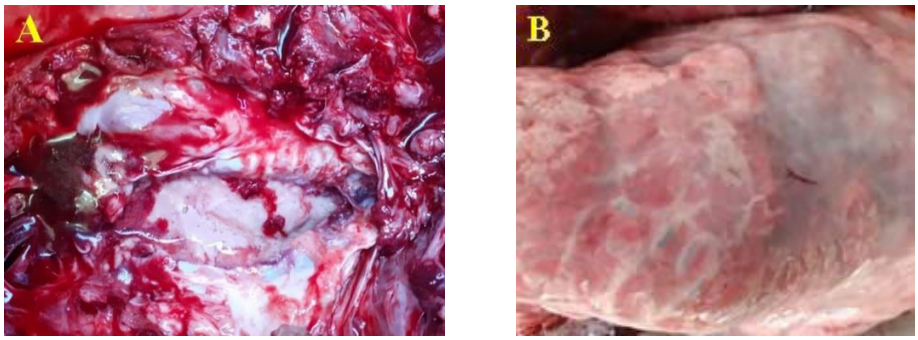

Fig. 1. Pulmonary edema (BPIV3) – A, subpleural emphysema (BPIV3) – B

In severe forms, well-expressed lobar bronchopneumonia has been found in the cranial and medial lobes (Fig. 2).

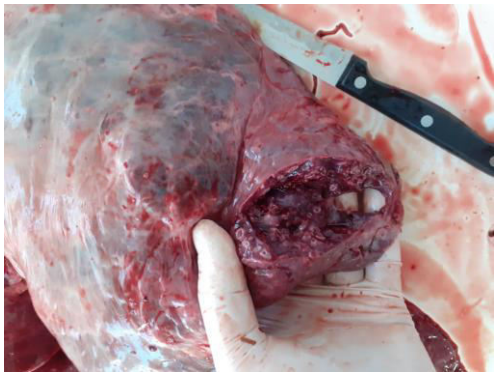

Fig. 2. Lobar bronchopneumonia (BPIV3)

Other authors have also reported atelectasis and cranial lobe compaction. They also described the presence of a mucopurulent exsudate, highlighted after organ sectioning (8, 16). Inflammation of the lymph nodes in the thoracic cavity,

along with pleurisy, has also been described. All these changes were found starting on the 4th day after the onset of the disease and up to 16 days after infection (8, 10).

Histopathologically, the presence of intranuclear acidophilic inclusions and cell syncytia in the alveoli (Fig. 3) (23) was found, which is a diagnostic element in BPIV3 infection.

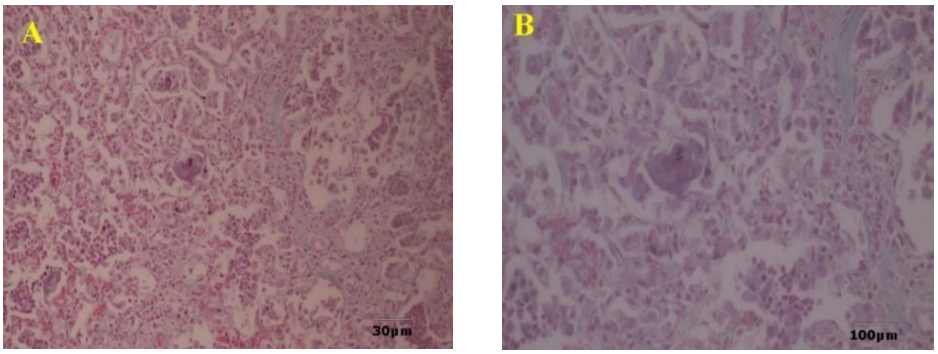

Fig. 3. Lobar bronchopneumonia - Giant-cellular syncytia (BPIV3), col. HEA, 20x ob. (A), 40x obj. (B)

In the literature, among the characteristic histopathological lesions are the bronchitis and alveolitis, accompanied by lysis of ciliated and non-ciliated epithelial cells, changes that occur within 24 hours of the actual infection (8). At 2-7 days, groups of eosinophils can be observed in the epithelium of the lung structures (bronchi, alveoli) (9, 10).

Macroscopic and microscopic lesions in complicated forms

In complicated forms with bacteria, fibrinous bronchopneumonia in the red hepatization phase (Fig. 4) and subepicardial suffusions (Fig. 5) were observed.

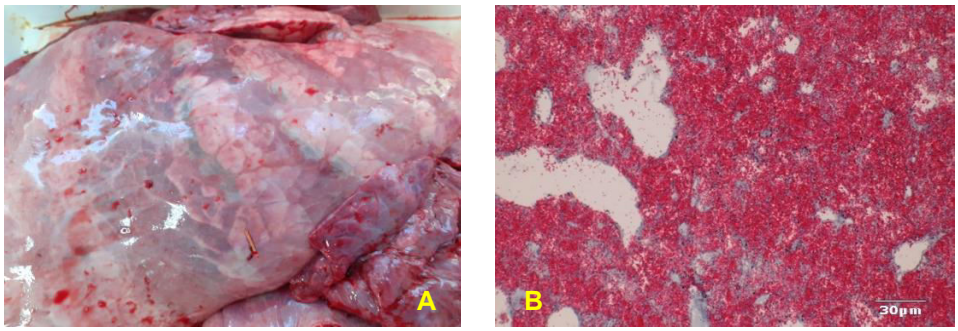

Fig. 4. Fibrinous bronchopneumonia - red hepatization phase: A. macroscopic aspect, B. microscopic aspect, col. HEA 20x

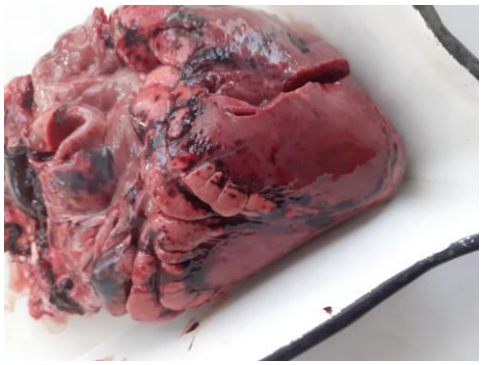

Fig. 5. Subepicardial suffusions

There was an accentuated distension of the lung in form of air bubbles in the interlobular space (alveolar emphysema) with dimensions about 3-4 cm. In some cases, vascular ruptures and interstitial hemorrhages caused by respiratory efforts were also observed.

At the level of the mediastinal and tracheobronchial lymph nodes, lesions of serous and hemorrhagic lymphoreticulitis in foci were observed (Fig. 6).

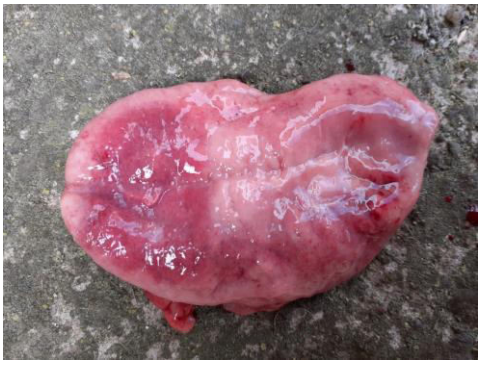

Fig. 6. Cattle tracheobronchial lymph node. Serohemorrhagic lymphoreticulitis
In the complicated forms, the proliferation of epithelial cells was found at the alveolar level, among with exsudate and dead cells.

The alveolar exsudate consists of fibrin network, numerous red blood cells, rare leukocytes and scaly alveolar cells. Secondary pulmonary emphysema was also found.

A dilation of marginal and perifollicular sinuses was observed in the lymph nodes due to serous exudate and the multiple congestion (Fig. 7).

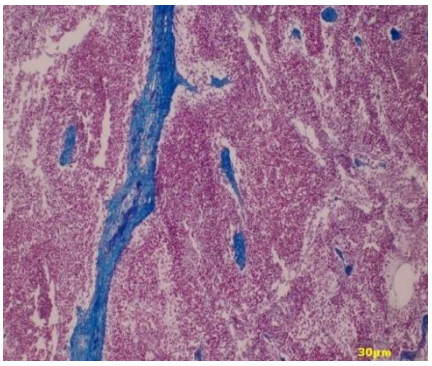

Fig. 7. Cattle tracheobronchial lymph node - serous lymphoreticulitis (reactive lymphoid follicles), col. HEA 20x

The results of the pathological examination are presented in the following table:

Table 1

Lungs and lymph nodes lesions

Nr. Crt	Lesion	Nr. corpses	%
1	Lobular bronchopneumonia	**17/30**	**56.66**
2	Fibrinous bronchopneumonia (red hepatization phase)	**7/30**	**23.33**
3	Lung allelectasis	**6/30**	**20**
4	Alveolar emphysema	**7/30**	**23.33**
5	Serous lymphoreticulitis	**12/30**	**40**
6	Hemorrhagic lymphoreticulitis in foci	**9/30**	**30**

The research is in line with existing data in the literature on macroscopic and microscopic lesions specific to BPI3 infection and complicated bacterial forms (3).

Conclusions

In the calve fattening farm, the infection with BPIV3 and *Pasteurella multocida* was confirmed and the source was represented by the newly calves introduced into the farm.

It is recommended that calves to be vaccinated at least 14 days before being introduced in the fattening farm.

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CONSIDERATIONS ABOUT INDUCED EXPERIMENTAL PERIODONTITIS IN RATS

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Summary

A lot of research has been done in the recent years to find a suitable therapy for periodontal disease and it has been shown to be very elaborate. This periodontal pathology is a widespread disease in the world and affects a large majority of the human and animal population so that periodontal disease needed to be experimentally induced in certain species, in order to successfully evaluate all the evolutionary factors involved in its development, but also to be able to test the effectiveness of various methods of therapy, some being experimental or ongoing implementation. Therefore this experimental framework was implemented in order to test a novel therapy represented by a biomaterial enriched with a photosensitiser and essential oils extract that has the potential of reversing the associated clinical and pathological symptoms.

Keywords: chronic inflamation, periodontium, alveolar bone loss, biomaterial.

Periodontitis is a chronic oral infectious and inflammatory disease caused by periodontopathogens which lead to the destruction of the tissues that support the teeth, respectively it causes the loss of the periodontal ligament and alveolar bone, and also could causes symptoms, such as gum bleeding, laxity of the teeth and plaque on teeth, could also develop an local inflammation as gingivitis (1, 11).

All these symptoms are largely considered to be the result from the response of a susceptible host to a microbial biofilm containing bacterial pathogens. Serum levels of pro-inflammatory cytokines, such as interleukin-1beta, interleukin-6, and tumor necrosis factor alpha (TNF), have been shown to be high in patients with severe chronic periodontitis (5, 17, 19).

There is a great diversity of bacterial species in the periodontal flora (9). The variation in the composition of the oral bacterial flora from one subject to another, as well as the variation of the host response to the interaction of bacterial species are some of the main reasons why the specific etiology of periodontal disease could not be clearly established (7, 8, 12). Bacteria is known to be the primary etiological agent of periodontal disease it has been estimated that more than 500 different bacterial species are able to attack an organism (4).

A big amount of the data already published on the etiology of periodontal disease comes from research on human diseases (15). Periodontitis in pets is a disease almost identical to that found in humans, in terms of progression and

clinical presentation (16). The accelerated rate of progression of the disease that has been reported in pets compared to humans may be caused by poor hygiene and lack of routine dental care (10). The disease can be influenced by some factors related to the environment, but also by the substrate and the genetic predisposition of each subject (2, 18).

Thereby, the aim of this study is represented by the experimental induction of a local inflammatory response in rats by placing a ligature on the first superior molar, to induce periodontitis which is demonstrated by histological aspects of an inflammatory response, presence of neutrophils, bone necrosis, rarefaction and alveolar bone loss (14, 20). Also, by experimental induction of the periodontitis, another aim of this study is to test the beneficial effects of biomaterials and photodynamic therapy, implementing an innovative method of alternative therapy in the treatment of periodontal disease in pets (3, 6). Also the avoidance of the side effects due to analgesic medication expressed by gastrointestinal disorders and the emergence of antibiotic resistance when using antibacterial medication could be a good start, because all of these represent ways of conventional methods of treating periodontitis (11).

Materials and methods

Medium weight female Wistar rats were used for this study. After the clinical exam, the appetite test was performed and then the rats were weighted forward. Body weight fluctuation was monitored daily after the placement of the ligature for 10 consecutive days.

Optimal accommodation conditions were ensured throughout the study, food in the form of standard granulated feed for rodents (from the Cantacusino Institute, Bucharest, Romania) and water ensured ad libitum, plus a low-calorie diet with a softer consistency for the first days post. The surgical procedures were performed under the effect of general injectable anesthesia.

The experimental protocol consisted in the application of a surgical technique in order to place the ligature on the molar (Fig. 1) which was performed under anesthesia, this was an injectable type for the application of the ligature and was performed by administering the following anesthetic substances: Xylazine, sol. inj. 8 mg / kg IM; Ketamine, sol. inj. 80 mg / kg IM (10).

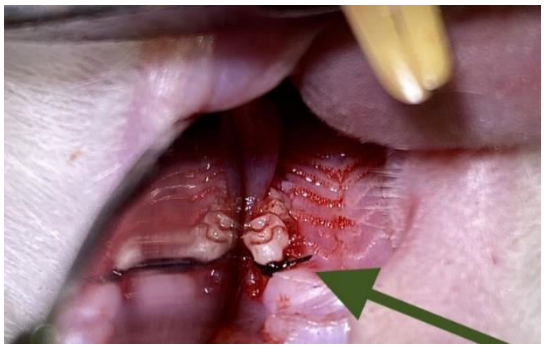

Fig. 1. The ligature at the base of the first left upper molar after performing a slight detachment of the gum, in the submarginal position and dislocation of the periodontal ligaments

To extract the ligature, the following were administered: Midazolam, sol. inj. 0.01-0.02 mg / kg SC and Ketamine, sol. inj. 80 mg / kg IM (10).

Deep narcosis with Isoflurane was used for euthanasia (for cervical / axo-atloid depinalization). If the side effects have significantly weakened the animal, it has been euthanized before the end of the study period, based on previously established criteria.

The study involved the induction of periodontitis in rats by ligating the first left upper molar (Fig. 2) under anesthesia and analgesia, which was subsequently treated with several regenerative therapy methods, such as the application of an antibacterial and osteoregenerative biomaterial, the application of a biomaterial with photosensitizing agents, as well as photodynamic therapy, in order to evaluate and appreciate the applicability of these techniques, as well as their effect, separately but also together (13).

In the first phase, all rats were weighed, then they were anesthetized according to the previously described protocol. The second phase involved the application of a ligature at the base of the first left upper molar (Fig. 2), previously performing a slight detachment of the gum, in the submarginal position and dislocation of the periodontal ligaments. 5-0 cotton or silk suture material was used.

After the application, the rats were weighed daily, and a pre-determined amount of special food was administered in order to check their appetite post-operatively. In the first two days, a 5% solution of Glucose injection was given if they did not eat any food at all and was also ensured their analgesia with an injectable solution of Tramadol.

The third stage took place one week after the ligation was applied, when the rats were anesthetized again, CT scan was performed and then the ligature placed on the molar was removed (Fig. 3).

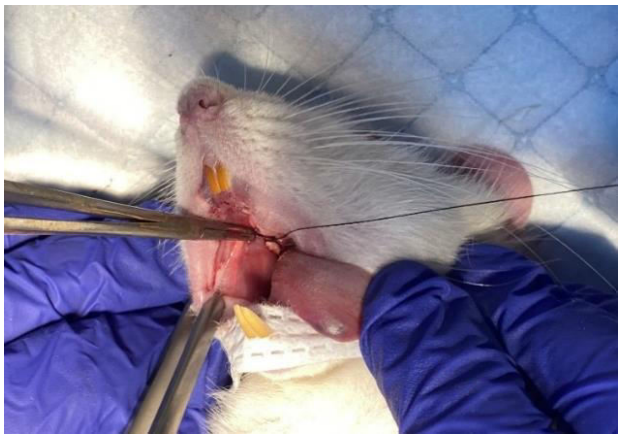

Fig. 2. The ligature at the base of the first left upper molar

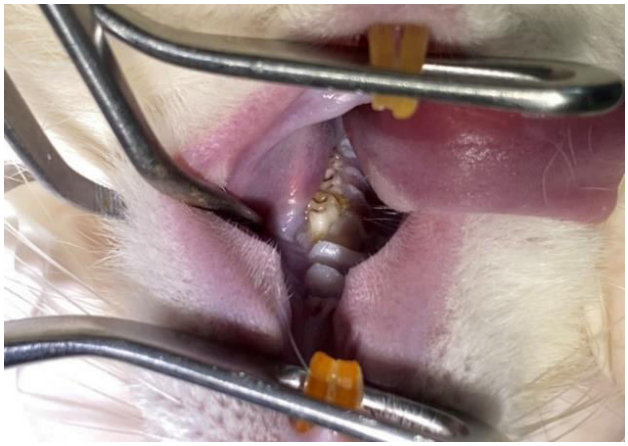

Fig. 3. Aspect of the molar after the removal of the ligature.
The bacterial plaque is visible

This stage was preceded by tooth scaling, root planning and soft movements of the molar in order to create an accumulation of plaque, flattening and the displacement of the gingival tissue (Fig. 4), thus provoking an inflammatory response, with the help of mini-curettes by distal-medial traction movements in the oral and lingual planes, repeated 10 times (15). The rats were euthanized one week after the ligation was removed, after which the left mandible was harvested for histopathological analysis in order to confirm the installation of periodontitis.

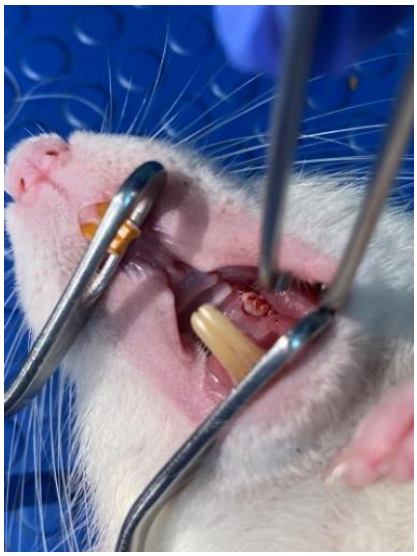

Fig. 4. Aspect of the molar after the tooth scaling, root planning and creating soft movements of the molar in order to create an accumulation of plaque

Results and discussions

Following the presentation of the protocol described above, it was applied to 10 rats in which we could see after 7 days the installation of periodontitis, respectively gingivitis was observed, in five subjects more moderate, in four others more acute, and in one subject gingivitis was reduced, so dental laxity was recorded in only 9 out of 10 rats. 6 of 10 rats lost weight, with variations ranging from 25 to 60 g. moderate amount (16).

After euthanasia, the rats were necropsied, and macroscopically, the yellowing of the molars, the movement in the alveolus and the reacted gum could be observed (Fig. 5).

Microscopically, lesions such as demineralization, thinning, and bone resorption could be seen in 7 rats, no lesion changes, and one rat had inflammatory lesions (Fig. 6).

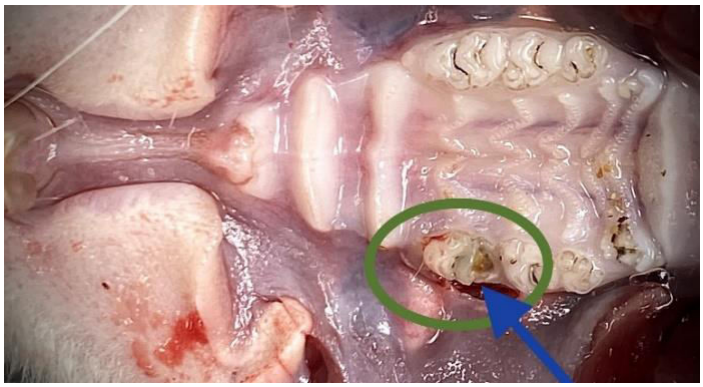

Fig. 5. Macroscopical aspects of periodontitis after euthanasia

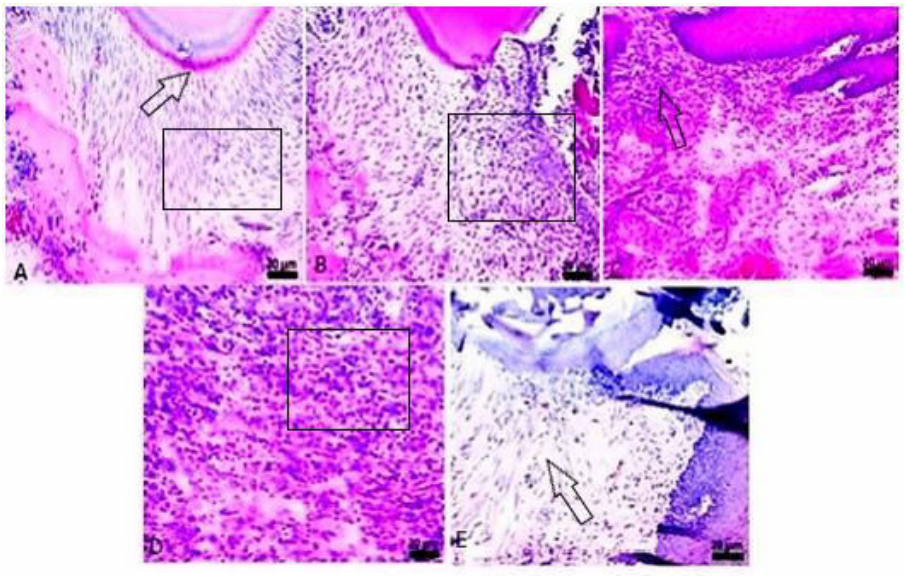

Fig. 6. Microscopically it can be observed an inflammatory response, neutrophils, bone necrosis, rarefaction and alveolar bone loss

Also, following the CT scan, changes in bone and periodontal tissue were also observed in 7 rats.

Conclusions

As periodontitis is one of the most common diseases in humans, many studies have used laboratory animals to investigate the pathogenesis of the disease. The placement of the ligature on the teeth has been proposed to obtain an experimental periodontitis faster than it occurs naturally.

This study shows that placing a cotton or silk thread around the cervical region of the upper left molar causes gingival inflammation and the first symptoms of periodontitis set in from the seventh day of the experiment. These inflammatory changes can be explained by a local release of inflammation mediators. In the present research, the aim is to evaluate the systemic biological implications of this pathology, investigating the correlation between periodontal disease, periodontal treatment and general health.

The tested procedure is able to provide all the key biological factors present in periodontal disease, whereas representing the adequate features for biomaterial testing domain. Another main aim of the research is to demonstrate the effectiveness of regenerative therapy with biomaterials, photosensitizing agents and photodynamic therapy, reversing all the effects of periodontitis induced by the initially placed ligature.

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EVOLUTION OF *BRUCELLA ABORTUS* INFECTION IN CATTLE IN EUROPE BETWEEN 2016 AND 2020

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Summary

Brucella infection is readily transmissible to humans, causing acute febrile illness (undulant fever) which may progress to a more chronic form and can also produce serious complications affecting the musculo-skeletal, cardiovascular, and central nervous systems. Infection is essentially acquired by the oral, respiratory, or conjunctival routes, but ingestion of raw milk products constitutes the main risk to the general public where the disease is endemic. The aim of this paper was to monitor the evolution of *Brucella abortus* infection in cattle in Europe in the last 5 years (2016-2020). Many countries from Europe are considered to be officially free of brucellosis (OFB) but, nevertheless, the EU's attention is directed to the countries where this disease is still evolving in countries such as Albania, Greece, Italy, North Macedonia, Portugal, Russia, and Serbia. The distribution of new outbreaks by year registered an increase in 2017 in the first semester (Jan-Jun) registering 675 new outbreaks of disease, then the distribution of new outbreaks decreases registering in the 2nd semester (Jul-Dec) 2019, 387 new outbreaks of the disease. The continuing presence of *Brucella abortus* infection in search high number of outbreaks requires epidemiosurveillance which be regularly reviewed and adapted accordingly.

Keywords: brucellosis, cattle, *Brucella abortus*.

There is an occupational risk to veterinarians, abattoir workers, and farmers who handle infected animals/carcasses and aborted fetuses or placentas. Brucellosis is also one of the most easily acquired laboratory infections, and all laboratory manipulations with live cultures or potentially infected with *Brucella spp*. Infection with *Brucella* in cattle is usually caused by biovars of *Brucella abortus* (4, 7, 9, 15, 18).

Brucellosis is a contagious disease of livestock with significant economic impact. The disease is caused by various bacteria of the family *Brucella*. Most *Brucella* species can infect other animal species as well brucellosis in cattle (*B. abortus*) in sheep and goats *(B. melitensis*) and in swine (*B. suis*) are listed in the World Organization for Animal Health (20) Terrestrial Animal Health Code and must be reported to the OIE. Brucellosis is a highly infectious zoonosis for humans (3, 6, 9).

The spread to humans most often occurs by drinking raw milk from infected animals. It causes a severely debilitating disease in people. Veterinarians, farmers, and abattoir workers are vulnerable to infection as they handle infected animals and aborted foetuses or placentae (13, 17). Human brucellosis is best prevented by

controlling the infection in animals. Pasteurization of milk from infected animals is an important way to reduce infection in humans (2, 18).

The continuing presence of *Brucella abortus* infection in search high number of outbreaks requires epidemiosurveillance which be regularly reviewed and adapted accordingly.

The infection spreads rapidly and causes many abortions in unvaccinated cattle. In a herd in which disease is endemic, an infected cow typically aborts only once after exposure; subsequent gestations and lactations appear normal. After exposure, cattle become bacteremic for a short period and develop agglutinins and other antibodies; some cattle resist infection, and a small percentage of infected cows spontaneously recover (11, 14, 17). A positive serum agglutination test usually precedes an abortion or normal parturition but may be delayed in ~15% of cows. The incubation period may be variable and is inversely related to the stage of gestation at the time of exposure (3, 6, 9). Organisms are shed in milk and uterine discharges, and the cow may become temporarily infertile. Bacteria may be found in the uterus during pregnancy, uterine involution, and infrequently, for a prolonged time in the nongravid uterus. Shedding from the vagina largely disappears with the cessation of fluids after parturition (12, 13). Some infected cows that were previously aborted shed brucellae from the uterus at subsequent normal parturitions. Organisms are shed in milk for a variable length of time—in most cattle for life. *B abortus* can frequently be isolated from secretions of nonlactating udders. Transmission may occur by artificial insemination when *Brucella*-contaminated semen is deposited in the uterus but, reportedly, not when deposited in the mid cervix (3, 7, 8, 15, 17, 19).

Materials and methods

The aim of this paper was to monitor the evolution of *Brucella abortus* infection in cattle in Europe in the last 5 years (2016-2020). The distribution of new outbreaks by year registered an increase in 2017 in the first semester (Jan-Jun) registering 675 new outbreaks of disease, then the distribution of new outbreaks decreases registering in the 2nd semester (Jul-Dec) 2019, 387 new outbreaks of the disease.

The epidemiological data were obtained from the site-ul Office International des Epizooties-World Animal Health Information System (20).

Results and discussions

From Fig. 1 and 2, it can be seen that most new disease outbreaks were in 2016 January-June 763 outbreaks, followed by 2017 -675 new disease outbreaks also in the first semester, and the least new disease outbreaks were in 2019-387 new disease outbreaks.

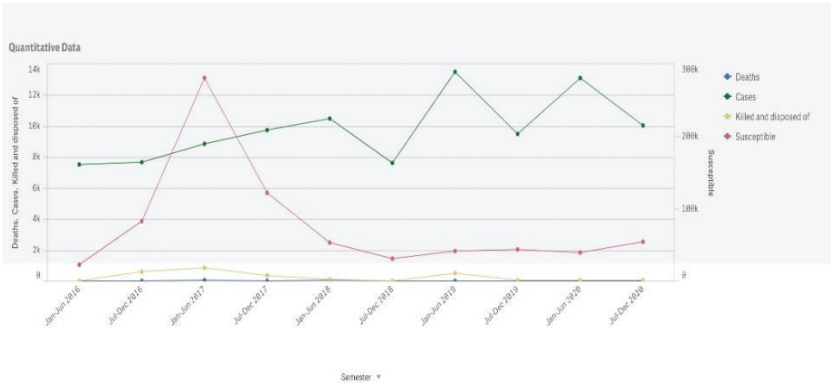

Fig. 1. Evolution of bovine brucellosis (20)

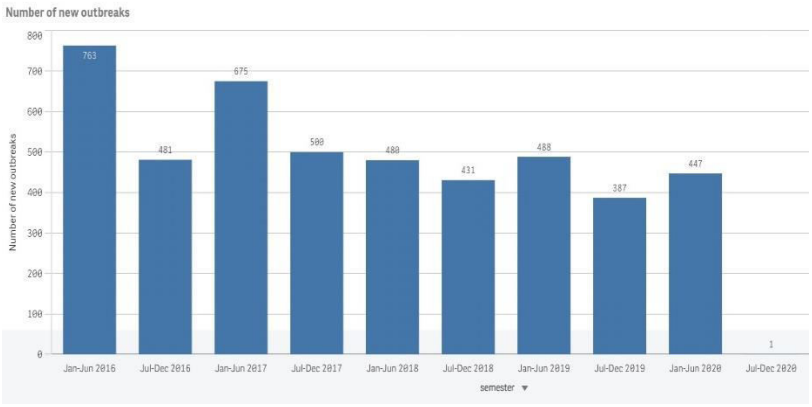

Fig. 2. Number of registered bovine brucellosis outbreaks (20)

Brucellosis in cows has a varied evolution in Europe between the years 2016-2020 (Fig. 3). The disease was reported and diagnosed in many countries in Europe, in both semesters taken in the study. In 2017 the disease was reported only in one semester in Albania and Spain. In 2018 also Albania together with Greece reported the disease in one semester, the rest of Bosnia, Italy, Macedonia, Portugal, Russia, and Serbia report it as present in both semesters. 2020 reports show that in countries like Russia and Italy the disease is still present throughout the year and in Spain only in one semester was brucellosis in cows present.

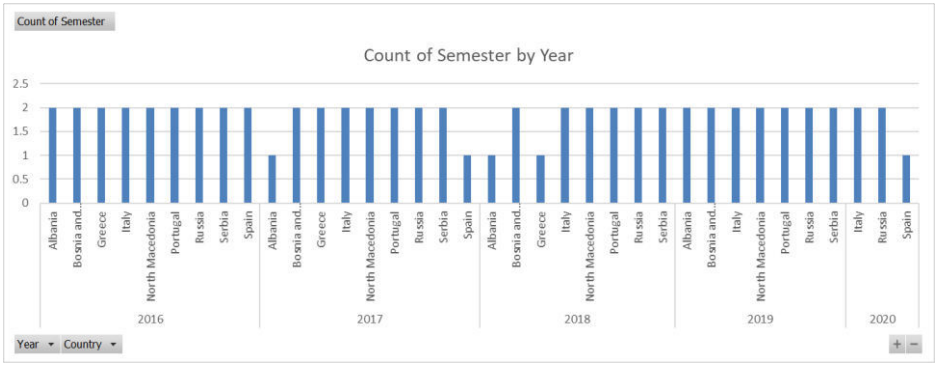

Fig. 3. Bovine brucellosis outbreaks – count by years and semesters (20)

Conclusions

According to the research, brucellosis is a zoonotic disease with an impact on humans. We know that it is transferred from animal to human through its products.

The evolution of *Brucella abortus* in Europe has had an oscillating evolution during the 5 years, registering a peak of the number of outbreaks in 2016 with 763 outbreaks, followed by a decreasing number of outbreaks.

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STUDY OF MATERNAL BEHAVIOUR AND NEWBORN DEVELOPMENT IN AFRICAN PYGMY HEDGEHOG (*ATELERIX ALBIVENTRIS*)

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Summary

The attempts to observe parturition and hoglets until a certain age often result in cannibalism or abandonment of the young by the female. This explains the lack of information regarding hedgehog births and hoglets development from parturition until the weaning age. The objective of this study is to describe the maternal behavior of the dams and the evolution of newborns of *Atelerix albiventris* from parturition until the weaning age. The study was conducted for a period of one year and a half on 3 mature African hedgehog females, with ages varying between 7- 18 months and on the hoglets that they gave birth to. As regarding the maternal behaviour, females were very protective and careful with their babies, but agresivity, selective abandonment or cannibalism were also seen. The females cross-fostering behaviour was seen as well. They were feeding and taking care of each other's hoglets like they were their own. Also, they were observed nursing the hoglets together. The hoglets were observed from birth until 6 weeks of age. The pups were weighed and measured and the changes in physical features were seen through visual inspection and noted. The average length at birth for the hoglets was 4.77±0.24 cm. The average body weight at birth was 9.2 g and at 5 weeks of age 84.5 g. Sexual determination of the hoglets was possible at 7-9 days of life. Fur apparition on face and on the body started at around 12-13 days of life. All the hedgehogs had their eyes opened between 14 and 19 days of age. Hoglets started consuming solid food at 4 weeks of life.

Keywords: *Atelerix albiventris*, hoglets, maternal behaviour, cross-fostering.

Hedgehogs belong to the order *Insectivora*, which includes the most primitive of all living placental mammals today (18). They are solitary and nocturnal animals (8).

Hedgehogs are polygamous and females are capable of breeding at a young age. Hedgehogs are polyestrous and the mating season occurs year – round (2).

The litter size is between one and nine with an average of 3-4 hoglets (10). When parturition is pending, the female hedgehog will gather available material in her mouth and use it to make a nest for herself and her offspring (4). Many females will not feed the night prior to parturition (20). Once young are born, females will continue to feed at night and spend the days in their nest caring for their young (13, 15). The female may cannibalize, kill, or abandon her newborn young if she is stressed or disturbed (1)**.** Observations of other hedgehog species report that during

birth the female may lie on her side or in sternal recumbency, or stand with hind legs apart. She licks the genital region periodically. During contractions the female strains and trembles; each contraction lasts several seconds. Young are born head first or feet first. Birth of the entire litter may take minutes to hours, and the mother may move about between births (5, 9). After each birth, she severs the umbilical cord, consumes the placenta and birth fluids and in the process, licks the neonate clean (6, 20). A female hedgehog is attentive to her offspring but may cannibalize or abandon them if she is stressed or disturbed after parturition (3). In case of absence of lactation or abandonment, the adoption of the hoglets from a dam with hoglets of approximately the same age is usually made with success (10).

Hoglets are born with closed eyes and ears, pink, hairless, with the spines located beneath the skin and surrounded by fluid to prevent damage to the mother's birth canal during parturition (11, 16). Their skin is edematous and covered with small bumps like the warts of a toad. Within a few hours of birth, the skin appears to "deflate" and flexible, soft white spines begin to emerge from the bumps. Newly born hedgehogs can vocalize loudly at birth. They can crawl, pull themselves around using their front limbs, and flip themselves over from their dorsum to their ventrum (15, 20). They typically nurse while lying on their backs and begin nursing soon after birth (20). Within 12–24 hours, the baby hedgehogs can walk and mothers will return a straying, vocalizing baby to the nest by carrying it in her mouth until it is about 4 weeks of age (15). By about 15 days of age, hedgehog's eyes and ears open and by about 21–25 days of age, they are eating solid food (12, 15).

Female hedgehogs are very attentive mothers, but males do not participate in the rearing of the youngsters at all (15). In fact, females should be separated from the males near the time of parturition as males have been known to eat newborn hedgehogs. Female hedgehogs with young can be surprisingly aggressive. One of the few times when a female hedgehog is likely to bite is when she has a litter of babies. She may hiss and snort and come out of the nest to attack if disturbed (17). This behavior may be seen to a lesser degree in females who are habituated to human handling and regular disturbance. Cross-fostering of abandoned or orphaned hedgehogs is usually successful as long as the mother's young are of a similar size to the baby being fostered (14, 20). In fact, when female hedgehogs are housed in groups, they have been observed to feed and care for each other's offspring (20).

Materials and methods

The study was conducted for a period of one year and a half on three mature African hedgehog females (Fig. 1) and their 17 hoglets resulted from four pregnancies. One of the females was monitored together with the offspring resulting from two pregnancies, and the other two females with offspring from one pregnancy each.

The females, together with their pups were living in plastic storage boxes, with stove pellets, paper litter, or sawdust as a substrate. Each storage box had a

wooden house, a running wheel, food and water dishes, and toys. Females were fed dry cat food, live mealworms and boiled chicken or scrambled eggs.

Maternal behavior monitoring was focused on most frequent interactions between a mother and her offspring, such as the time spent in contact, licking/grooming and nursing of hoglets. These interactions were observed through the video camera which was installed in the wooden house. Also the females were seen outside of their nest interacting with their hoglets. Two of the three females were put together, in the same cage, 4 weeks after giving birth along with their hoglets, in order to evaluate ,,the cross-fostering behavior''.

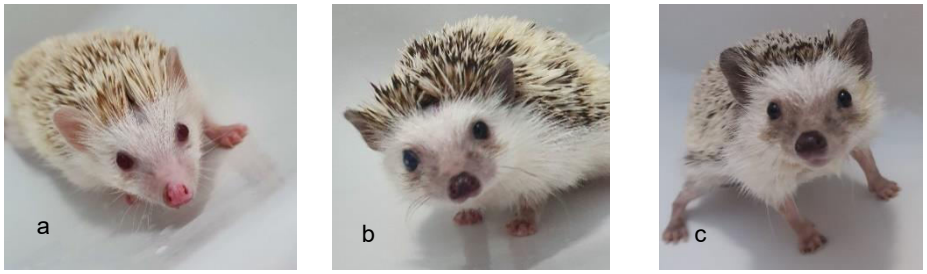


Fig. 1. The three African hedgehog females included in the study

Postnatally, the evolution of hoglets from birth to weaning was monitored. The following aspects were tracked: fur apparition and quills development, the age when the hoglets opened their eyes, the age when the sex determination was possible, the onset age of solid food consumption, hoglets behavior**,** body length and body weight dynamics. Direct and indirect visual inspection of the hoglets body surface, eyes and external genitalia was performed daily. Hoglets were also photographed daily in order to identify certain details. Hoglets behavior was observed on video camera (Xiaomi Mi Home Security Camera Basic 1080P), outside of the nest, and also when they were handled. Body weight of the hoglets was determined, using a MYRIA MY4185 Kitchen scale.

The hoglets were manipulated with gloved hands which were previously impregnated with each female's bedding smell. This action was performed in order to not transfer foreign smells to the hoglets within the weighing process.

Results and discussions

Maternal behavior monitoring reveals a first mother-offspring interaction during parturition: all the females were seen consuming the fetal membranes from each hoglet and clean the newborns. Female provides protection to hoglets, takes care of them and stays with them most of the time. The most frequent position that females embrace for nursing is the lateral recumbency. They were also seen nursing in dorsoventral recumbency. After nursing the offspring, the females were stimulating

urination and defecation in hoglets by licking their anogenital region. This interaction is very important in stimulating the excretion process of the newborns. One of the females manifested her protective behavior by blocking the entrance from her wodden house with the food dishes and every time the hoglets needed to be weighed she used to huff loudly and cover them with her body so they can't be taken from the nest (Fig. 2) Another female would huff loudly and throw herself in order to protect her pups. In order to manipulate the hoglets the dam had to receive food to distract her attention. After each measuring and weighing of the hoglets, the female investigated them cautiously (Fig. 3).

Fig. 2. Female who blocked her wooden cage entrance with the food dish because she felt threatened

Fig. 3. Female examining her pups after they were taken from the cage to be weighed

Abandonment and cannibalism of one newborn was observed in case of one female that gave birth to 4 hoglets. Three of the hoglets were females and the one that she abandoned couldn't be identified because of the small age. The abandoned hoglet was weighed and measured and put back in the nest but the dam ate it after that. There is a possibility that this behavior was generated by the handling of newborns. Likewise, the abandonment may be based on other unspecified considerations.

However, others have noted that if females are used to regular handling, they are less likely to cannibalize their young. Some individual females are simply more tolerant of disturbance than others (20). Nevertheless, when breeding hedgehogs in captivity, it has been recommended that the dam and newborn litter should not be disturbed prior to about 7 days post-parturition (12, 14).

The two females who were put in the same cage with their offspring, were observed taking care of each other's pups after they were put together. Both females were seen nursing all the pups, no matter who they belonged to. They were also spotted nursing their hoglets together (Fig. 4).

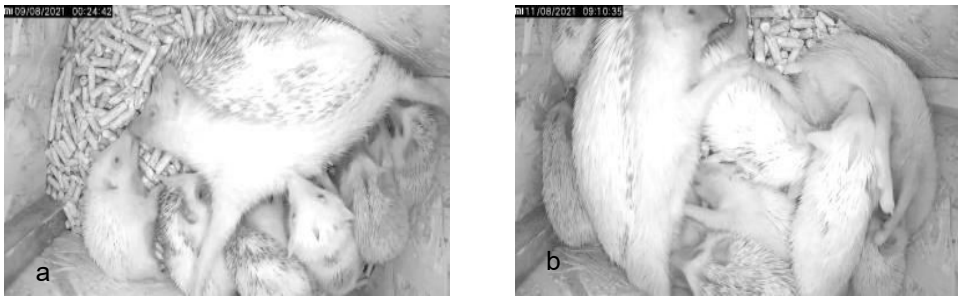


Fig. 4. Females nursing the hoglets together

In the female that have had two pregnancies, we noticed that for the first gestation the dam had just one hoglet and she was really protective with it for the first four weeks (Fig. 5). She used to leave the nest and run on the wheel with her pup inside her mouth. When the hoglet was 4 weeks it was found with a hematoma in her eye. After one week she had an injury in her left ear, following the appearance of hematomas in her limbs. The female was seen for more times trying to attack the pup so they were separated when the hoglet was 5 weeks old. At the second gestation the female gave birth to six hoglets. She was very affectionate and protective with them (Fig. 6) and didn't show any sign of agression like she did in her first gestation.

Fig. 5. Female with her hoglet from first gestation trying to put it back in the wooden house

Fig. 6. Female at the second gestation nursing her 15 days old hoglets

The mortality rates of newborn until weaning was of 12% (from 17 newborns, 2 died), including the cannibalized case. In captivity, the mortality rates of captive born hedgehogs may be as high as 25%. Mortality rates of newborns in the wild are not that much better: it has been calculated that 20% of wild-born hedgehogs may die before weaning (13).

The results of monitoring the evolution of hoglets from birth to weaning are presented below.

Hoglets were born blind, hairless, with pink skin with small bumps under it. The initial white quills emerged at 1.5-2 hours after birth. At 2 days of life the skin started to change its colour for the hedgehogs with dark spines, and stayed pink for the ones that would have white quills in the future. At approximately 12 days of age fur started to appear on ventral surface of hoglets bodies, and on the limbs. At 18 days of life the fur was seen also on the hoglets faces. (Fig. 7).

a. Two hours after birth | b. Six hours after birth | c. One day of life | d. One week of life

e. Two weeks of life | f. Three weeks of life | g. Four weeks of life | h. Six weeks of life

Fig. 7. Dynamics of quills development

In specialized literature it was mentioned that within 24 hours, the full length of these first spines should be apparent (about 4.9–5.5 mm) (15, 20). The initial spines are unpigmented, flattened, and shorter than those of the adult. A second set

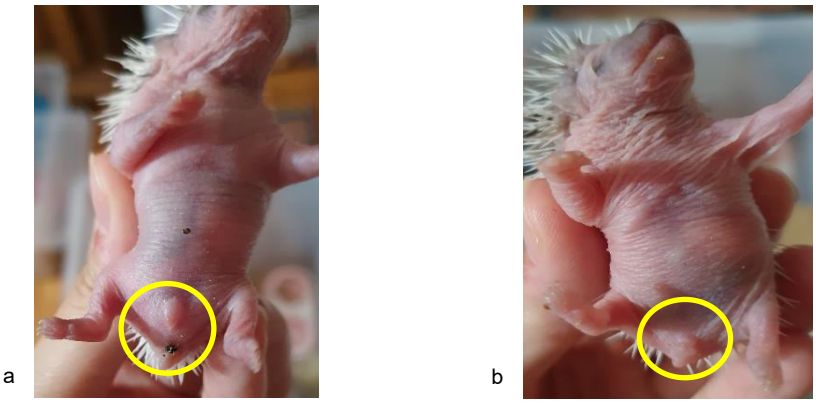
of spines, which more closely resembles those of the adult, emerges within 2–3 days of birth (19). Most hedgehogs have a covering of short fur by the time they are 2 weeks old. However, *Atelerix albiventris* babies have been noted to have some hair on their abdomen as early as a day of age (20).

The sex of the hoglets could be determined by visual inspection of the external genitalia from 7-9 days of life. External landmarks (distance between the anus and the external genitalia, and also the appearance of the genitalia which is more prominent in males) can be used to distinguish male versus female hoglets (Fig.8). The sex of the hoglets was determined only for 15 out of 17 newborns. The other two died and the sex could not be identified due to small age. Sex ratio male: female was 1:4, of the 15 hoglets, 3 were males and 12 were females (Table 1).

Table 1

Ratio between male and female hoglets from this study

Female	No. of gestations	No. of hoglets/ 1st gestation	No. of hoglets/ 2nd gestation	Total no. of hoglets/female	Ratio male/female
Female 1	1	4	-	4	0:3
Female 2	1	6	-	6	2:3
Female 3	2	1	6	7	1:6
Total	4	11	6	17	3:12



a b

Fig. 8. Sexual determination using direct visual inspection in 7 days old hoglets

At 14-19 days of age all the hedgehogs had their eyes opened (Fig. 9). At 4 weeks of life the hoglets started consuming the kitten kibbles that their mom used to eat.

Some of the hoglets were separated from their mothers around 5 weeks, while others were naturally weaned between 5 weeks and 6 weeks and a half. Last day the hoglets were observed suckling was at 46 days old.

In one population of captive hedgehogs, the oldest young ever observed still suckling was 44 days of age (12). Typically, in captive-bred hedgehogs, weaning will begin at 4–6 weeks of age (20) and most young hedgehogs are fully weaned by 40–44 days (12). However, some may be weaned as late as 10 weeks (7).

Fig. 9. Hoglet whith his eyes opened in day 14 of life

Table 2

Results for the length monitoring of the hoglets

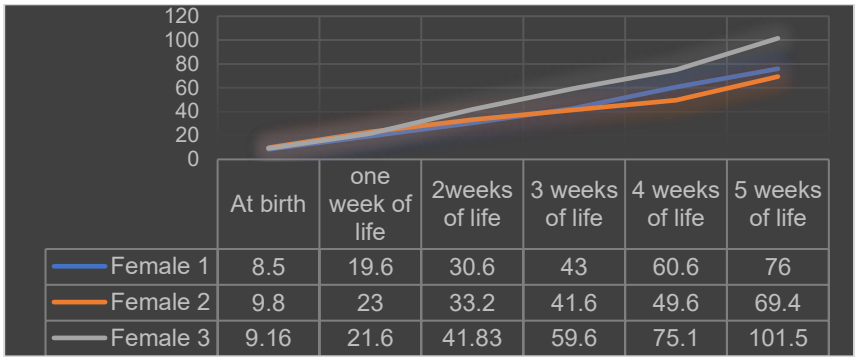
Hoglets age	At birth	One week of life	2 weeks of life	3 weeks of life	4 weeks of life	5 weeks of life
Length of the hoglets (cm)	4.77±0.24	6.81±0.24	7.3±0.24	11±0.31	12.3±0.4	13.2±0.4

Length of the hoglets at birth was 4.77±0.24 cm, and the length at 5 weeks of life measured 13.2±0.4 cm. It can be observed (Table 2) that the hoglets tripled their length from birth until 5 weeks of life. Also the most significant increase in length was observed from 2nd to 3rd week of life (almost 4 cm in one week).

The values noted for monitoring of the body weight of the hoglets reveal a gradual increase from birth until 5 weeks of life. It registers differences between the hoglets average weight of each female, varying between 76 and 101.5 g (Fig. 10).

For the female who had two gestations, for the first one hoglet, and for the second one 6 hoglets, it was observed that the hoglet from the first gestation measured 150 g at 4 weeks, while the average weight for the other litter of the female was 75.1 g at the same age. This fact emphasizes the influence of the number of products of conception on the body weight of the hoglets.

The increase in the average body weight from all the hoglets belonging to the 3 females had the highest values in the first week of life (the multiplication rate was x 2.35) (Fig. 11).



	At birth	one week of life	2weeks of life	3 weeks of life	4 weeks of life	5 weeks of life
Female 1	8.5	19.6	30.6	43	60.6	76
Female 2	9.8	23	33.2	41.6	49.6	69.4
Female 3	9.16	21.6	41.83	59.6	75.1	101.5

Fig. 10. The average body weight (grams) for each female's hoglets from birth until 5 weeks of life

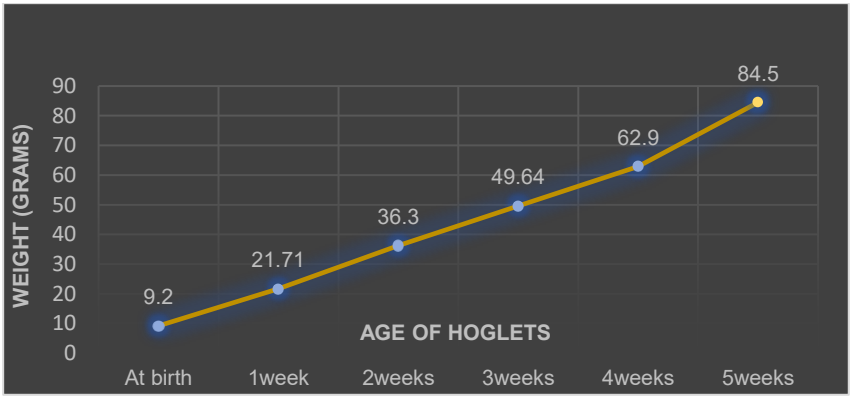


Fig. 11. The average body weight for all the pups belonging to all 3 females from birth until 5 weeks of life

Hoglets behaviour Although they interacted well with each other, young hoglets didn't show any specific play behavior like in other species, but they were observed offering affection to their mothers (Fig. 12).

A unique behavior met in African Hedgehog is the self-annointing behavior which was highlighted at hoglets since 12 days of life (Fig. 13).

Fig. 12. Hoglets being affectionate with their mothers

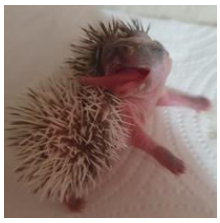


Fig. 13. Self-annointing in a 12 days old hoglet

Conclusions

Females of African hedgehog are attentive and protective mothers, but they abandoned, and cannibalized pups when they felt threatened. Cross-fostering behavior was observed.

The initial white quills of the hoglets emerged at 1.5-2 hours after parturition. All the hedgehogs had their eyes opened between 14 and 19 days of age.

Sexual determination of the hoglets was possible at 7-9 days of life, based on the anogenital distance and proeminence of the genitalia.

Values registered at the morphometric analysis revealed that the body length of the hoglets at birth was 4.77±0.24 cm and the length at 5 weeks measured 13.2±0.4 cm. Average weight for the hoglets at birth was 9.2 g, and at 5 weeks measured 84.5 g.

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STUDY REGARDING THE PATHOGENETIC, CLINICAL, AND DIAGNOSTIC COORDINATES IN LIVER AND SPLENIC CYSTIC DISEASES IN COMPANION ANIMALS

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Summary

Hepatic cysts are defined as cystic formations, bounded by a capsule with epithelium, which can be congenital or acquired, solitary or multiple. Splenic cysts are uncommon and are often found incidentally on imaging examinations. In general, it is estimated that the pathogenesis is extremely diverse and sometimes unknown, and clinically, expressions are often reduced and uncharacteristic or absent. In this paper are presented the results of our study regarding the incidence, feature and diagnostic coordinates of the liver and splenic cysts in dogs and cats.

Keywords: splenic cysts, liver cysts, dog, cat.

Hepatic cysts are defined as cystic formations, bounded by a capsule with epithelium, which can be congenital or acquired, solitary or multiple (3, 9). Congenital cysts are associated with polycystic kidney disease, having a genetic character, and acquired cysts are usually solitary (7, 17, 18).

It is known that in mammals the primary simple hepatic cysts are congenital and appear as a result of defects in the formation of bile ducts (ducts), and secondary (acquired or pseudocysts) occur after some inflammation, infestation or even following other processes / phenomena of lesion and / or mechano-traumatic (12, 13, 14, 19).

Hepatic cysts may progress asymptomatically, being detected at a routine clinical and paraclinical examination, without being accompanied by manifestations of organ failure, or may express mild or severe forms of hepatic dysfunction, correlated with an associated pathology, depending on the size of the cyst (1, 4, 11).

Splenic cysts are uncommon and are often found incidentally on imaging examinations (8, 10, 15).

There are four types of splenic cysts: primary or congenital cysts and secondary cysts represented by posttraumatic pseudocysts, hydatid cysts due to *Echinococcus granulosus* infestation and intrasplenic pancreatic pseudocysts (5, 20).

Congenital cysts may develop asymptomatically or cause pain or symptoms associated with gastric compression, often being attributed to cysts larger than 8 cm (16, 20).

Secondary cysts

Post-traumatic pseudocyst is an epithelial-lined cyst formed by the organization, liquefaction, resorption, and encapsulation of a splenic hematoma, subsequent blood absorption, and persistence of a false wall (10).

The ultrasound characteristics for cystic lesions are round-ovoid shaped lesions/structures, single or multiple, with variable dimensions, being delimited by a fine hyperechoic wall and a transonic/ anechoic content, accompanied distally by the presence of specific artefact, phenomenon - distal enhancement (16, 17).

Materials and methods

This research was conducted within the Emergency Hospital of the Faculty of Veterinary Medicine Bucharest and within the Vet Medical Consulting, between April 2020 and October 2021, been identified cystic lesions in a number of 76 patients.

Haematological and biochemical screening tests and ultrasonographic exams were performed on Sonoscape and Esoate My Lab30 ultrasound with a convex probe with a frequency of 5-8 MHz as an algorithm for diagnosis (Table 1).

Table1

Hepatic and splenic cysts in cats

NUMBER OF TOTAL PATIENTS *(n=76)*			
CATS (n=39)			
Renal localization	***(n=28)***	***(n=4)*** ***(n=24)***	**M=27** **F=12**
Hepatic localization	***(n=8)***		
Splenic localization	***(n=3)***		

Table 2

Hepatic and splenic cysts in dogs

NUMBER OF TOTAL PATIENTS (n=76)			
DOGS (*n=37*)			
Renal localization	***(n=7)***		
Hepatic localization	***(n=4)***		M=**12** F = **6**
Splenic localization	***(n=7)***		
Prostatic localization	***n=19***	***n=11*** ***n=8***	M=**19**

Results and discussions

In 76 patients, cystic lesions were recorded in 39 cats, 28 with renal localization (single or multiple cysts), 8 with hepatic localization and 3 with splenic localization.

In dogs, 37 patients registered cystic lesions, 19 with prostatic localization (single/multiple), 7 renal, 4 hepatic and 7 splenic.

The hematological and biochemical screening performed did not revealed detectable and/or correlated changes with the cystic lesions presence, that were diagnosed using ultrasound technique.

The ultrasound feature of cystic lesions are diagnosed mainly based on the characteristic appearance, round-ovoid shaped lesions/structures, single or multiple, of variable dimensions, delimited by a fine hyperechoic wall and a transonic/ anechoic content, accompanied distally by the presence of specific artefact, phenomenon - distal enhancement (Fig. 1 – 6).

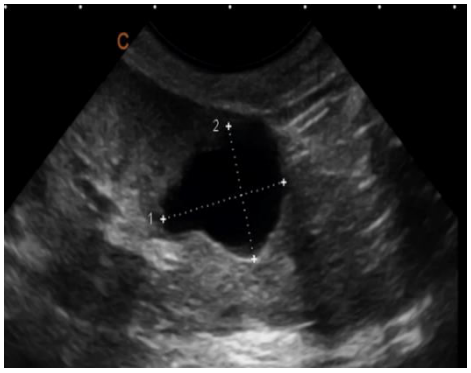

Fig. 1. Splenic cyst in dog

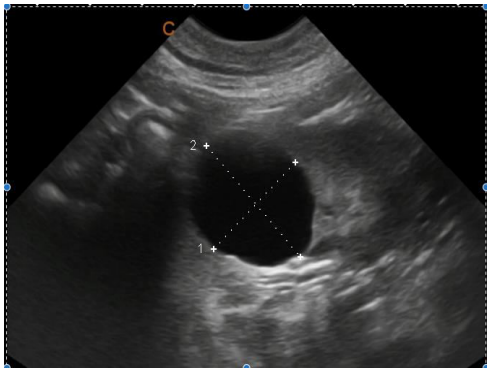

Fig. 2. Splenic cyst in cat

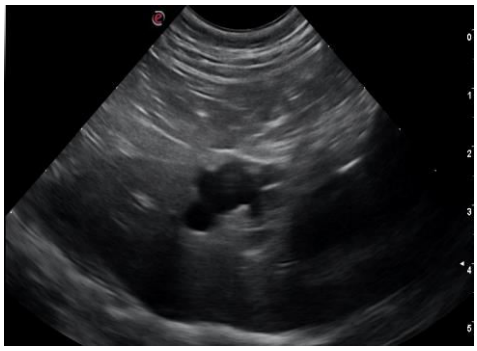

Fig. 3. Liver cysts in cat

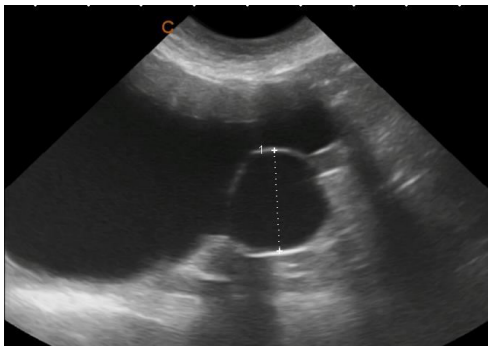

Fig. 4. Liver cyst in dog

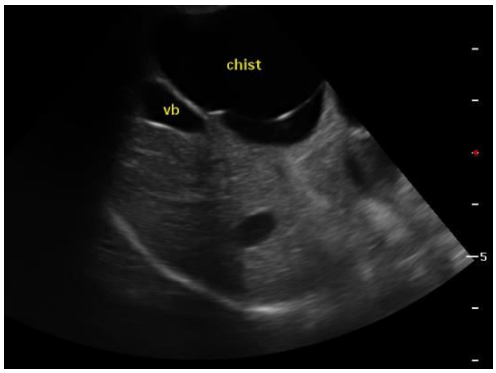

Fig. 5. Liver cysts in cat

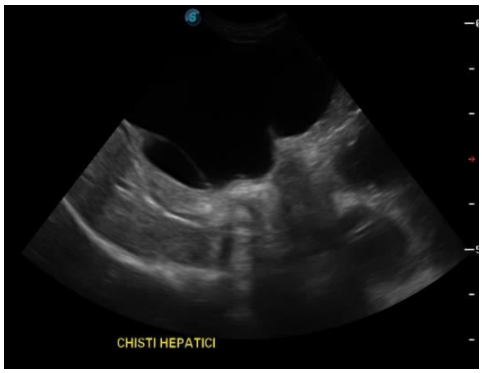

Fig. 6. Liver cyst in dog

Conclusions

Due to its non-invasive nature and high accuracy, ultrasound examination can be considered the imagisthique diagnostic method with the highest degree of specificity, considered golden standard in the identification and assessment (in dynamics) of cysts in liver and spleen.

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UNCOMMON BRONCHOPNEUMOPATHY IN A STRAY CAT – CASE REPORT

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Summary

An old stray cat presented with severe respiratory distress, lethargy and poor appetite. At the physical examination the body temperature was normal, respiration rate 62/minute and the heart rate 138 bpm without significant audible cardiac murmurs. Radiographic images showed the presence of the bronchopneumopathy with a diffuse bronchoalveolar pattern throughout the lung fields, characterized by bronchial and alveolar changes and a poorly structured interstitial pattern. Serum biochemical profile revealed creatinine 1.6 mg/dl, urea 151.11 mg/dl, total proteins 8.37 g/dl, albumin 2.69 g/dl, TGP 114.68 U/l, TGO 42.08 U/l, GGT 0.32 U/l, ALP 38.30 U/l, glucose 110.74, triglycerides 56.12 mg/dl, phosphorus 4.46 mg/dl. Hematological blood analysis showed a mild non-regenerative anemia (5.51 M/µl red blood cells) and leukocytosis (30.45 k/ µl white blood cells). Based on these findings the cat was diagnosed with stage I chronic kidney disease. A further fecal investigation by Baermann technique led to the identification of an *Aelurostrongylus abstrusus* severe infection. These findings indicate that aelurostrongylosis should be included in the differential diagnosis of feline respiratory distress even in non-endemic regions and should perform appropriate diagnostics procedures in the presence of compatible symptoms.
Keywords: cat, bronchopneumopathy, *Aelurostrongylus abstrusus*, lungworm.

Chronic bronchopulmonary disorders in cats represent a group of airway and alveolar space disorders. Clinical signs are consider being due, in part, to underlying airway obstruction. Factors contributing to the development of airway obstruction can include development of airway inflammation and mucosal edema, development of airway smooth muscle hypertrophy and constriction, and excessive production or retention of pulmonary secretions. Bronchopulmonary disorders are often caused by direct infection with bacteria, viruses, parasites or fungi as well as by immune-mediated reactions or inhalation of irritants or toxic substances. In cats, the most common clinical signs associated with chronic bronchopulmonary disorders are dyspnea, cough and lethargy (2, 4). Investigating a cat with respiratory symptoms to establish the etiological diagnosis can be a challenge. Cats with bronchopulmonary disease often have hematological and biochemical profiles that are unremarkable or show non-specific change but are important to exclude any other systemic diseases that might be affecting the respiratory system. Hematologic abnormalities can include an erythrocytosis from chronic hypoxia, leukocytosis with infections, eosinophillia with parasitic infections or asthma. Thoracic radiography is a valuable noninvasive tool for investigating respiratory

disease. Although diagnostic specificity may be low and rarely can a clinician form a definitive diagnosis based only on radiographs, thoracic radiography is very useful tool in differential list (2, 4).

The present case report underlines the importance of appropriate diagnostic methods before excluding the infection with lungworms even in non-endemic areas or when other diseases are suspected.

Materials and methods

An old stray cat presented with severe respiratory distress, lethargy and poor appetite. At the physical examination the body temperature was normal, respiration rate 62/minute and the heart rate 138 bpm without significant audible cardiac murmurs. After the clinical evaluation, the cat was submitted for cardio-pulmonary radiographic images, blood analysis (serum biochemical profile, cells blood count) and urinalysis. An enzyme-linked immunosorbent essay for FIV/FeLV was performed and it was positive for feline immunodeficiency. Based on the thoracal radiographic findings, a Baermann coproparasitological exam was performed and it was positive for A *abstrusus*. Following the diagnosis, a single administration of milbemicin oxime 16 mg/praziquantel 40 mg (Milbemax®, Elanco) and doxycicline (10 mg/kg s.i.d. for 10 days) was given without any improvement on clinical condition or the result of coproparasitological examination. One week after milbemicin oxime/praziquantel was given it was applied spot-on imidacloprid/moxidectin (Advocate®, Bayer) and repeated 14 days later. After the second administration of the spot-on treatment, coproparasitological exam was negative but there was no improvement on clinical state of the cat. Further, it was initiated a treatment with prednisolone (2.5 mg b.i.d. for 10 days). Due to a major improvement of the clinical state of the patient, prednisolone was reduced to 2.5 mg s.i.d and eventually to 1.25 mg s.i.d.

Results and discussions

In Table 1 and 2 are presented the hematological and biochemical profiles of the cat. Hematological blood analysis showed a mild non-regenerative anemia (5.51 M/µl red blood cells) and leukocytosis (30.45 k/ µl white blood cells).

Serum biochemical profile revealed increased levels of creatinine 1.6 mg/dl, urea 151.11 mg/dl, total proteins 8.37 g/dl, TGP 114.68 U/l. Urinalysis showed moderate proteinuria and a low concentrated urine (specific gravity 1020). Based on these findings the cat was diagnosed with stage I chronic kidney disease (IRIS CKD Staging Guidelines).

The initial radiographic findings, presented in the Fig. 1 (a, b) on lateral and dorsoventral recumbencies showed the presence of a bronchopneumopathy with a mixed pulmonary pattern that was mainly interstitial and poorly defined.

Table 1

The results of the sanguine hematological and biochemical parameters

RBC (M/µl)	5.51	Total proteins (g/dl)	8.37
Haematocrit (%)	26.7	Albumin (g/dl)	2.69
Haemoglobin (g/dl)	9.3	TGP (U/l)	114.68
MCV (fL)	48.5	TGO (U/l)	42.08
MCH (pg)	16.9	GGT (U/l)	0.32
MCHC (g/dl)	34.8	Creatinine (mg/dl)	1.6
Reticulocyte (K/ µl)	21.5	Urea (mg/dl)	151.11
WBC (K/ µl)	30.54	Glucose (mg/dl)	110.74
Neutrophils (K/ µl)	21.49	Triglycerides (mg/dl)	56.12
Lymphocytes (K/ µl)	6.39	Phosphorus (mg/dl)	4.46
Monocytes (K/ µl)	1.34	Potassium (mmol/l)	4.51
Eosinophils (K/ µl)	1.21	Sodium (mmol/l)	159.32
Basophils (K/ µl)	0.11		
Platelets (K/ µl)	280		

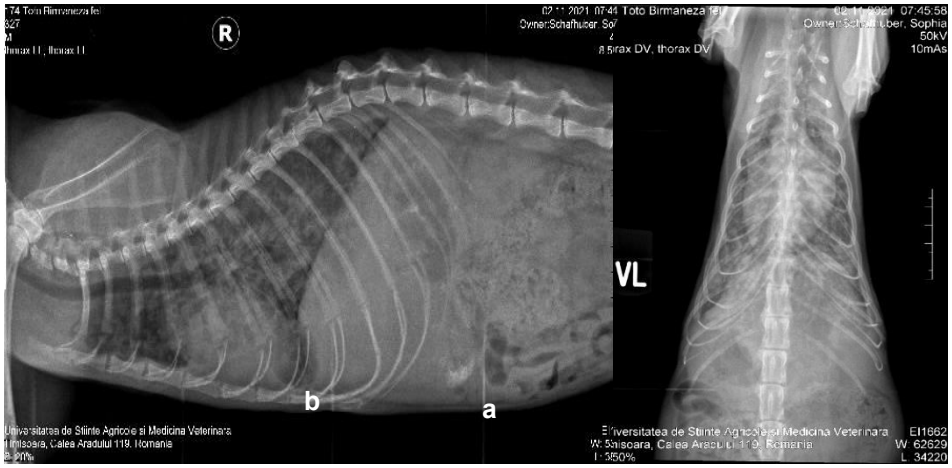

Fig. 1. Initial lateral (a) and dorsoventral (b) thoracic radiographs before treatment

Also, several small nodules with both poorly defined and well-defined margins, as well as bronchial patterns in the form of ring-like opacities (“doughnuts”) and parallel lines (“tram lines”) were identified. Changes were more severe in the caudodorsal lung field.

The results of fecal examination by Baermann technique confirmed severe infection with *A abstrusus* which explains all the clinical symptoms and the pulmonary area lesions. The administration of milbemycin oxime and doxycycline did not yield significant improvement of neither the clinical state or the result of a repeated Baermann method examination, which led to the use of imidacloprid/moxidectin spot-on (Advocate®, Bayer). Furthermore,

coproparasitologic examinations following the first administration of imidacloprid/moxidectin successfully decreased the number of larvae found and a second administration 2 weeks later resulted in the complete elimination of larvae. The radiographic examinations were repeated 7 weeks after the initial findings and are presented in Fig. 2 (a, b). Although there is a slight improvement on radiographs taken 7 weeks later, the aspect of bronchopneumopathy is maintained. It can be noticed the same mixed pulmonary pattern that was mainly interstitial and poorly defined.

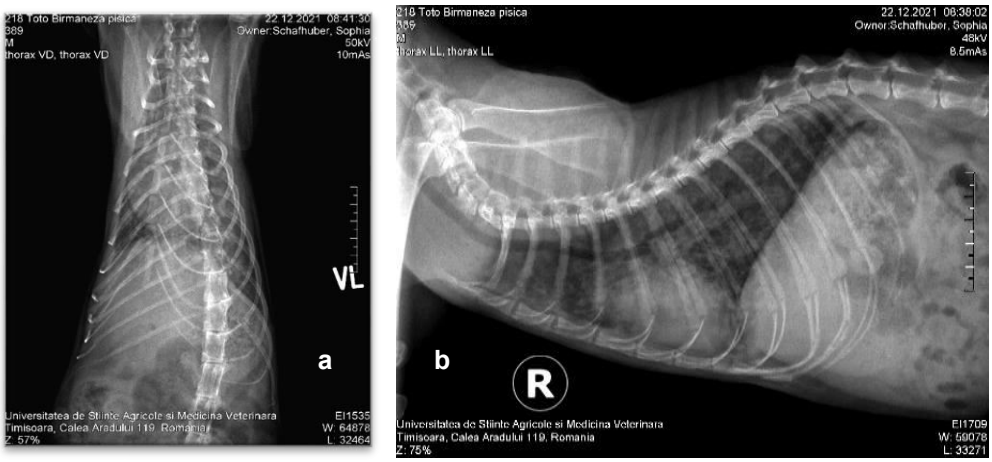

Fig. 2. Follow-up dorsoventral (a) and lateral (b) radiographs after 7 weeks from diagnosis and treatment

Mircean et al. (9) described the prevalence of *A abstrusus* in Romania in 2010 to be at 5.6 % in domestic cats. The species perpetuates itself by elimination of stage 1 larvae through feces which require a gastropod intermediate host in order to advance to stages 2 and 3. Ingestion of either the intermediate host, or more commonly, the ingestion of a paratenic host such as a mouse or bird, leads to the infection of the felid definitive host. Time elapsed between infection and evolution of adult stages within the lung approximates 6 weeks. Mild infections typically take an asymptomatic course, however heavy infections may present with cough, dyspnea, tachycardia, hemorrhages and may even be fatal. Definitive diagnosis is made by fecal examination using the Baermann technique which is considered the gold standard method in diagnosis of lungworms (8, 12).

A. abstrusus has been described to be persistent and difficult to treat by different authors (8, 14, 15). Fenbendazol is a frequent choice and authors such as Bowman (1) as well as Sanchez et al. (10) among others has been describing its efficacy (1, 3, 10, 11). A more recent study conducted by Iannino et al. (6) in 2012 however tested the use of Selamectin on forty-two cats and found its efficacy to be

between 59 % and 98 %, however two administrations were necessary in order to yield these results. Similar results have been described in earlier years concluding a partial efficacy of macrocyclic lactones (1, 3, 7). The use of imidacloprid 10%/Moxidectin 1% (Advocate®, Bayer) showed surprisingly good results in a study conducted by Traversa et al. (2009). Twelve cats were given a single dose of Advocate® according to the instructions of the label and all 12 cats were found to have a reduction of the larvae count per gram feces of 100%. A comparison was made at the same time with an equal number of cats (12) who received 50 mg/kg bodyweight of fenbendazole *per os* for three consecutive days. The fenbendazol group was found to have a slightly lesser reduction of larvae count per gram feces with a result of 99.28 % (13, 14). The efficacy in treatment and prevention of Advocate was proven by another study in 2020 were a 100 % prevention of maturation of experimentally inoculated stage 3 larvae was achieved and the treatment of adult *A abstrusus* had a high efficacy when administered once and a 100% efficacy when administered 3 times in monthly intervals (5).

Conclusions

Infection with *Aelurostrongylus abstrusus* can conduct to verminous bronhpneumopathy and represent an important differential diagnosis for cats with history of respiratory distress, cough or wheezing.

Aelurostrongylosis should be included in the differential diagnosis of feline respiratory distress even in non-endemic regions and appropriate diagnostic procedures should be performed in the presence of compatible symptoms.

In this case, oral milbemycin oxime was not effective in the treatment of *Aelurostrongylus abstrusus.*

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RABBIT COCCIDIOSIS IN *LEPUS EUROPAEUS* AND *ORYCTOLAGUS CUNICULUS* IN EUROPE: ETIOLOGICAL AND EPIDEMIOLOGICAL REVIEW

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Summary

Coccidiosis is one of the most important protozoon diseases caused by Eimeria species. Rabbits are highly susceptible to coccidiosis, especially after weaning time. The prevalence of this disease in European countries is between 3 and 100%. Coccidiosis has been reported in both domestic (*Oryctolagus cuniculus*) and wild (*Lepus europaeus*) rabbits. One of the most pathogenic species of eimeria is *Eimeria stiedae* which parasitises the liver of domestic, wild and laboratory rabbits. All other eimeria species found in rabbits are found in the intestine. Of these gut-localised species, the most pathogenic are *Eimeria intestinalis* and *Eimeria flavescens*, and the least pathogenic, affecting young rabbits, are *Eimeria irresidua, Eimeria matsubayashii, Eimeria magna* and *Eimeria perforans.* The most common species of eimeria found in European countries in *Oryctolagus cuniculus* are: *Eimeria coecicola, Eimeria exigua, Eimeria flavescens, Eimeria intestinalis, Eimeria irresidua, Eimeria magna, Eimeria media, Eimeria perforans, Eimeria piriformis, Eimeria roobroucki, Eimeria stiedae and Eimeria vejdovskyi*, and the most common eimeria species in *Lepus europaeus* are: *Eimeria coquelinae, Eimeria europaea, Eimeria hungarica, Eimeria leporis, Eimeria macrosculpta, Eimeria stefanskii, Eimeria taillliezi, Eimeria inquirendae, Eimeria belorussica.* However, till now, only 11 species have been described: *E. magna, E. media, E. irresidua, E. flavescens, E. intestinalis, E. coecicola, E. piriformis, E. perforans, E. exigua, E. vejdovsky* and *E. stiedae*. This article summarizes the current knowledge on the rabbit coccidia and the diseases they cause. Various aspects, such as etiology, life cycle, localization in the host, epidemiology, pathology and prevalence are discussed.

Keywords: rabbit, coccidiosis, Europe, *Oryctolagus cuniculus, Lepus europaeus*.

Rabbits are unique animals, easily adaptable to laboratory conditions as experimental animals in research, but also as pets. As well as being very important and necessary as laboratory animals, they are bred for a variety of commercial purposes including meat and fur.

Coccidiosis caused by *Eimeria* species not only causes significant economic losses in the poultry industry worldwide, but is also responsible for major losses in the rabbit industry with a high incidence of morbidity and mortality (1).

Coccidiosis is one of the most important protozoon diseases caused by *Eimeria* species, evolving in three clinical forms: hepatic, intestinal and oto-rhino-pharyngeal. Rabbits are highly susceptible to coccidiosis, especially after weaning time.

Eimeriosis has been reported in both domestic (*Oryctolagus cuniculus*) and wild (*Lepus europaeus*) rabbits.

The aim of this paper is to review the current knowledge about coccidiosis in rabbits in Europe.

Etiology

Coccidia was among the first single-celled organisms, identified by Antoine van Leeuwenhoek in 1674, when he observed "corpuscles" in the gall bladder of a domestic rabbit. Since then, literature documenting the development of knowledge about coccidiosis in rabbits has become widespread. The study of coccidiosis in rabbits began in earnest after the work of Hake (13), who first described the pathology in the liver and duodenum of domestic rabbits (9).

Coccidia of the genus *Eimeria* are members of the *Apicomplexa* Phylum, *Eimeriidae* family (10).

The most common species of *Eimeria* found in European countries in *Oryctolagus cuniculus* (domestic rabbit – Fig. 1) are: *Eimeria coecicola*, *Eimeria exigua*, *Eimeria flavescens*, *Eimeria intestinalis*, *Eimeria irresidua*, *Eimeria magna*, *Eimeria media*, *Eimeria perforans*, *Eimeria piriformis*, *Eimeria roobroucki*, *Eimeria stiedae* and *Eimeria vejdovskyi.* This species are described în Table 1 (10).

The *Eimeria* species identified in European countries in *Lepus europaeus* (wild rabbit – Fig. 2) are: *Eimeria coquelinae*, *Eimeria europaea*, *Eimeria hungarica*, *Eimeria leporis*, *Eimeria macrosculpta, Eimeria stefanskii*, *Eimeria tailliezi*, *Eimeria inquirendae* and *Eimeria belorussica*. The characteristics of this species are described în Table 2 (10).

However, till now, only 11 species have been described: *E. magna, E. media, E. irresidua, E. flavescens, E. intestinalis, E. coecicola, E. piriformis, E. perforans, E. exigua, E. vejdovsky* causing intestinal coccidiosis in rabbits and *E. stiedae* causing hepatic coccidiosis in the liver.

Fig. 1. *Oryctolagus cuniculus*

Fig. 2. *Lepus europaeus*

Table 1.

Species of *Eimeria* found in *Oryctolagus cuniculus* in Europe (according to Boch & Supperer, modified) (4)

No. crt.	Species	Characteristics	Localization	Geographic distribution
1.	*Eimeria coecicola* (Cheissin, 1947) (5)	Oocyst shape: elongate-ellipsoidal or cylindroidal L x W (µm): 35,5 x 19,5 (23-40 x 15-21)	Ileum, cecum	France, Hungary, Poland
2.	*Eimeria exigua* (Yakimoff, 1934) (36)	Oocyst shape: subspheroidal L x W (µm): 14 x 13 (12-21 x 9-18)	Small intestine	France, Hungary, Italy, Poland, Spain
3.	*Eimeria flavescens* (Marotel & Guilhon, 1941) (20)	Oocyst shape: ovoidal L x W (µm): 31,7 x 21,4 (25 – 37 x 14 – 24)	Jejunum, ileum, cecum, colon	Belgium, France, England, Poland
4.	*Eimeria intestinalis* (Cheissin, 1948) (6)	Oocyst shape: broadly pear-shaped to ovoidal L x W: 27 x 18 (21 – 36 x 15 – 21)	Ileum, cecum, colon	Belgium, France, Hungary, Italy, Portugal
5.	*Eimeria irresidua* (Kessel & Jankiewicz, 1931) (14)	Oocyst shape: ellipsoidal to slightly ovoidal L x W (µm): 38,3 x 23,8 (25 – 49 x 16 – 28)	Jejunum, ileum	Belgium, Czech Republic, France, Italy, Poland, Portugal
6.	*Eimeria magna* (Perard, 1925b) (27)	Oocyst shape: ovoidal to ellipsoidal L x W (µm): 35 x 24 (27 – 41 x 17 – 29)	Jejunum, ileum	Belgium, Czech Republic, France, Germany, Italy, Poland, Portugal
7.	*Eimeria media* (Kessel & Jankiewicz, 1931) (14)	Oocyst shape: ovoidal to ellipsoidal L x W (µm): 31.2 x 18.5 (27 – 36 x15 – 22)	Jejunum, ileum, cecum, colon	Belgium, Finland, France, Italy, Poland, Portugal, Sweden

8.	*Eimeria perforans* (Leuckart, 1879) (16)	Oocyst shape: ovoidal, ellipsoidal, cylindroidal, or subspheroidal L x W (µm): 23 x 14 (15 – 31 x 11 – 20)	Duodenum, jejunum	Belgium, Czech Republic, England, Finland, France, Germany, Italy, Poland, Portugal
9.	*Eimeria piriformis* (Kotlan & Pospesch, 1934) (15)	Oocyst shape: piriform, often asymmetrical L x W (µm): 29 x 18 (26 – 33 x 17 – 21)	Cecum, colon	Belgium, Czech Republic, France, Hungary; Poland, Portugal, Spain
10.	*Eimeria roobroucki* (Gres, Marchandeau & Landau, 2002) (12)	Oocyst shape: ellipsoidal L x W (µm): 55 x 33,7		France
11.	*Eimeria vejdovskyi* (Pakandl, 1988) (23)	Oocyst shape: ellipsoidal, asymmetrical L x W (µm): 32,9 x 19,2 (30 – 37 x 18 – 21)	Ileum	Czech Republic, Slovakia
12.	*Eimeria stiedae* (Lindemann, 1865) (17)	Oocyst shape: elongate-ovoidal to ellipsoidal; L x W (µm): 37 x 20 (31 – 42 x 17 – 25)	Biliary ducts	Belgium, Czech Republic, Finland, France, Germany, Hungary, Italy, Netherlands, Poland, Portugal, Sweden, Switzerland

Table 2.

Species of *Eimeria* found in *Lepus europaeus* in Europe (according to Pellérdy, modified) (25)

No. crt.	Species	Characteristics	Localization	Geographic distribution
1.	*Eimeria tailliezi* (Aoutil, Bertani, Bordes, Snounou, Chabaud & Landau, 2005) (2)	Oocyst shape: elongate ellipsoidal L x W (µm): 42 x 23 (40 - 43.5 x 22 - 24.3)		France
2.	*Eimeria babatica* (Aoutil, Bertani, Bordes, Snounou, Chabaud & Landau, 2005) (2)	Oocyst shape: elongate - ovoidal L x W (µm): 25 x 19 (20 - 30 x 16 - 21)		Hungary
3.	*Eimeria coquelinae* (Aoutil, Bertani, Bordes, Snounou, Chabaud & Landau, 2005) (2)	Oocyst shape: ellipsoidal L x W (µm): 35 x 23 (32 - 39 x 20 - 26)		France
4.	*Eimeria europaea* (Pellerdy, 1956) (26)	Oocyst shape: ellipsoidal or fusiform		Austria, Bulgaria, Czech Republic, France, Germany,

		L x W (µm): 32 x 18 (26 - 34 x 15 - 20)		Hungary, Italy, Poland, Spain, Switzerland
5.	*Eimeria hungarica* (Pellerdy, 1956) (26)	Oocyst shape: spheroidal to subspheroidal L x W (µm): 14 x 13 (12 - 15 x 11 - 14)	Small intestine	Hungary
6.	*Eimeria leporis* (Nieschulz, 1923) (21)	Oocyst shape: cylindroidal L x W (µm): 32 x 16 (26 - 38 x 13 - 20)	Small intestine	The Netherlands
7.	*Eimeria macrosculpta* (Sugar, 1979) (34)	Oocyst shape: cylindroidal or elongate-ellipsoidal L x W (µm): 46 x 26 (40 - 50 x 25 - 33)	Large intestine	France, Hungary
8.	*Eimeria belorussica* (Litvenkova, 1969) (18)	Oocyst shape: ovoidal to ellipsoidal L x W (µm): 26 - 28 x 14 - 16;		Belarus
9.	*Eimeria stefanskii* (Pastuszko, 1961a) (24)	Oocyst shape: ellipsoidal to ovoidal L x W (µm): 59 - 68 x 32 - 37	Small intestine	Austria, Poland, Czech Republic, Slovakia

Life cycle

The life cycle (29), in general, is the same for all Eimeria species.

However, host specificity, site of development, prepatent periods (can last between 4 and 14 days to complete), and pathogenicity vary from species to species.

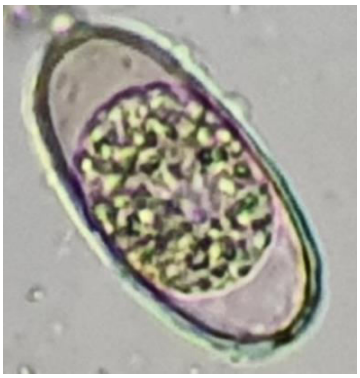

Fig. 3. Immature *Eimeria* oocyst

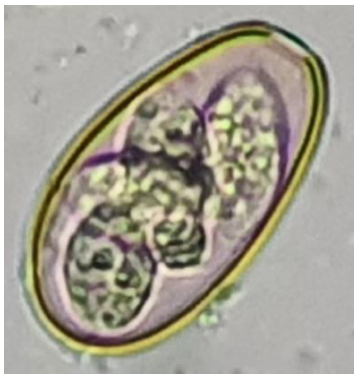

Fig. 4. Infestant *Eimeria* oocyst

Infestation of rabbits occurs when they clean their contaminated fur or by ingesting feces contaminated with eimeria oocysts or consuming contaminated feed. Reaching in the digestive tract, oocysts are mechanically and enzymatically digested

and sporozoites are released. The released sporozoites invade the epithelial cells lining the intestinal wall and initiate merogony (asexual multiplication). During merogony, sporozoites multiply asexually to form about 100.000 merozoites per sporozoite, depending on the *Eimeria* species. When the merozoites are mature, the host cell ruptures and releases merozoites, which in turn will penetrate another cell to repeat the merogony again. The number of merogony generations is different depending on the species and can vary from two to four generations. The last generation of merozoites to enter the host cells transforms into gametes, during gametogony (sexual multiplication). Most of them develop into macrogametes, the 'female' cell involved in sexual reproduction. The remaining gametes divide into thousands of microgametes, the 'male' cell involved in the sexual reproduction. When the microgametes are mature, they leave the host cell and penetrate a new host cell containing macrogamete and fertilization proceeds. As the newly formed zygote begins to mature it develops its resistant oocyst wall. When the oocyst wall is fully formed, it will leave the host cell and be eliminated into the outside environment with the feces (Fig. 3). Once in the outside environment sporogony occurs, to become fully mature (Fig. 4). Inside the oocyst four sporocysts develop, ready to infest a new host (8, 29).

Epidemiology

Coccidiosis in rabbits primarily is a disease affecting young rabbits, while adult animals are carriers, thus eliminating oocysts into the environment. Rabbits become infested by ingesting sporulated oocysts with feed or water. The severity of the disease depends on the number of oocysts ingested, the species of coccidia involved, the habitat of the rabbit, and the immune and nutritional status of each rabbit (25).

The disease occurs most commonly in intensively reared animals, but can also occur in well-cared-for rabbits.

The sources of contamination are sick rabbits, those that have been through the disease, and adults carrying parasites, contaminating bedding, cages and feed (33).

Intestinal coccidiosis typically affects young rabbits from 6 weeks to 5 months of age. Animals over 6 months are resistant. Most intestinal species develop in the small intestine, while only *E. flavescens* and *E. piriformis* complete their development in the cecum and colon, respectively. The disease develops more frequently in spring, with overcrowding, lack of ventilation and wet bedding, inadequate feeding and microclimate contributing to the outbreaks (8).

Hepatic coccidiosis, caused only by *E. stiedae*, affects rabbits of all ages when the parasite develops in the bile ducts of the liver, which enlarge excessively,

interfering with liver function. However, rabbits from 2-3 weeks to 4-5 months of age are most receptive, with resistance increasing significantly after 4 months (7).

In general, during both intestinal and liver coccidiosis, the normal function of the infested cells is inhibited, the cells are hypertrophied, and eventually die, thus the growth of rabbits is hampered by side effects in the kidneys and liver.

The resistance of oocysts in the environment is high, they can survive in bedding and shaded places for up to 150 days, and at temperatures of 80°C they are destroyed in 10 seconds (8).

Pathology

Liver coccidiosis has been known for many years. Coccidia that invade the biliary duct epithelium after infestation cause proliferation after a few days. The biliary ducts are very distended and obstructed by detritus, and a cauliflower-like epithelial proliferation is observed on the surface of the lamina. The parenchyma is surrounded by infiltrative inflammatory cell nodules. The parenchyma damaged by coccidia is replaced by fibrous tissue, and new capillaries grow to replace those destroyed by the parasite (19).

Intestinal eimeriosis, depending on their localization, attack the epithelial cells and give rise to an inflammatory reaction in the surrounding tissues. The extent of inflammatory reactions depends on the species of parasite, the number of coccidia invading the intestinal wall, and the pathogenic or non-pathogenic nature of the parasites, thus coccidia have been divided into five groups according to their pathogenicity: highly pathogenic (*E. intestinalis* and *E. flavescens*), pathogenic (*E. media, E. magna, E. piriformis* and *E. irresidua*), low pathogenic (*E. perforans, E. exigua* and *E. vejdovskyi*), non-pathogenic (*E. coecicola*).

The most pathogenic species, *E. intestinalis* and *E. flavescens* parasitize in the crypts and microvilli of the small intestine, respectively in the cecum, morphopathologically showing severe atrophy of the microvilli, inflammation of the intestinal mucosa, epithelial cells being mostly denuded.

Even species known to be non-pathogenic can cause serious digestive disorders and mucosal denudation if the infestation is massive (22, 28).

Prevalence

Parasitic infestations are a common feature among wild rabbits, but also among rabbits raised in human households. The impact of parasitic infestations on host health is closely related to environmental parameters such as weather conditions, food supply, population density, other parasites.

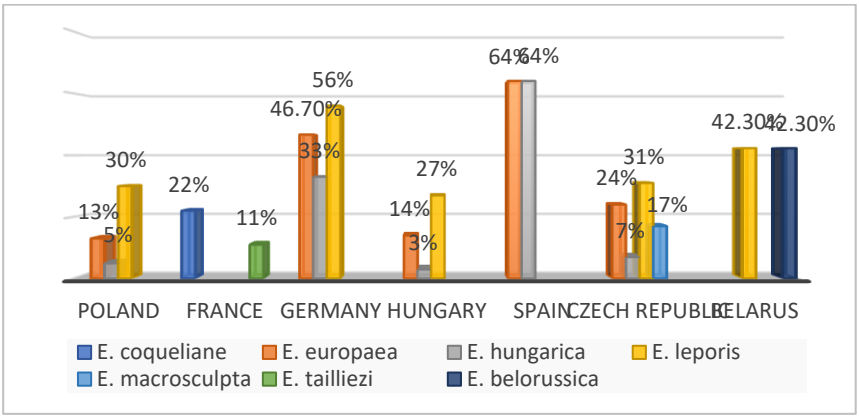

Fig. 5. Prevalence of *Eimeria* spp. in *Lepus europaeus* in European countries

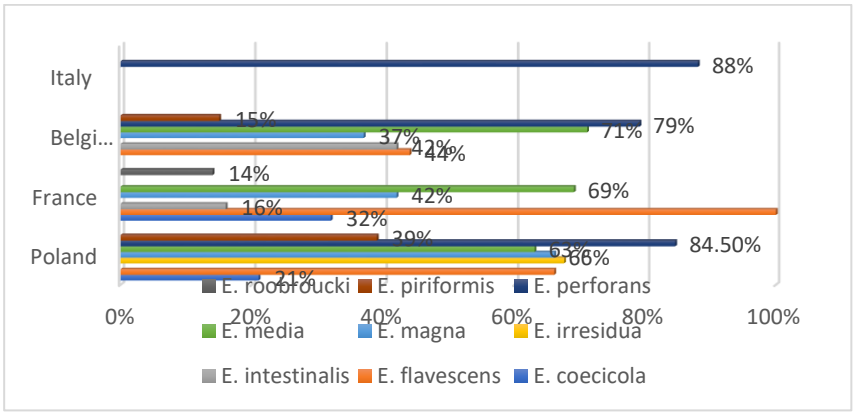

Fig. 6. Prevalence of *Eimeria* spp. in *Oryctolagus cuniculus* in European countries

A number of *Eimeria* species causing coccidiosis in domestic and wild rabbits have been reported from many countries of Europa. The prevalence of *Eimeria* species found in *Lepus europaeus* (Fig. 5) ranges from 3% to 64% and in *Oryctolagus cuniculus* (Fig. 6) from 14% to 100% (10).

The highest prevalence of *Eimeria* spp. infestations in Lepus europaeus was observed in Eimeria europaea in Spain (32) and Germany (11), 66% and 46.7% respectively, but also in *Eimeria hungarica* (64%) and *Eimeria leporis* (56%), while the lowest prevalence was identified in *Eimeria hungarica* in Hungary (3%), Poland (3) (5%) and the Czech Republic (7%).

Regarding *Eimeria* species identified in Europe, the highest prevalence was observed in France for *Eimeria flavescens* (100%), followed by *Eimeria perforans* (88% in Italy (30, 31), 84.5% in Poland (35) and 79% in Belgium), and the lowest prevalence was observed for *Eimeria roobroucki* (14%) and *Eimeria intestinalis* (16%) in France (2), followed by *Eimeria piriformis* (15%) in Belgium.

Conclusions

The most common species of *Eimeria* in Europe that infest *Lepus europaeus* are *E. leporis* (Germany, Hungary, Czech Republic, Belarus and Poland), *E. hungarica* (Poland, Germany, Hungary, Spain and Czech Republic) and *E. europaea* (Poland, Germany, Hungary, Spain and Czech Republic), and the most frequent species that parasitize *Oryctolagus cuniculus* are: *E. perforans* (Italy, Belgium and Poland), *E. flavescens* (Belgium, France and Poland) and *E. media* (Belgium, France and Poland).

It can be observed that there are no common species of *Eimeria* which parasitize both domestic and wild rabbits, but it can be noted that there are countries where parasitism is caused by several species.

The highest and lowest prevalence in *Oryctolagus cuniculus* was observed in France in *E. flavescens* (100%) and *E. roobroucki* (14%), respectively, and the highest and lowest prevalence in *Lepus europaeus* was found in *E. hungarica* in Spain (64%) and Hungary (3%).

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CASE REPORT OF HYDROCEPHALUS IN KITTEN POSSIBLY CAUSED BY INBREEDING

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Summary

Hydrocephalus is a neurologic disorder characterised by an excessive accumulation of cerebrospinal fluid (CSF) in the brain ventricles and arachnoid cavity caused by excessive production or inadequate re-absorption into the bloodstream by blockage in the ventricular system. CSF accumulation enlarges the brain leading to increased pressure in the skull, followed by thinning of the cranial bones and compression of the brain, including brain atrophy and a variety of symptoms, some of which may be life threatening. Hydrocephalus is classified mainly as communicating when it does not involve a blockage in the ventricular system or noncommunicating when it involves a blockage. Factors that lead to an increased incidence of this disease may be related to gene mutations, disease processes (infectious agents, brain tumor, hemorrhage, or inflamation), or teratogenic substances (griseofulvin). This anomaly can be found in all species of animals such as cows, horses, exotic animals, birds, dogs or cats as we will describe later on in this paper. Our case report involves a 1 year old female domestic cat in its first litter, unvaccinated, and uncertain date of any antiparasitic treatment applied. The cat has received a treatment to prevent the heat cycle, more likely in the period that she already has been mounted. The cat was brought to the veterinarian due to the impossibility of normal parturition. A Caesarean section was performed and five kittens were obtained, two of them had hydrocephalus, one of them fetal anasarca and two were normal and viable. Anamnesis indicated that the kittens are the result of an inbreeding between the sister and the brother. Taking into account the inbreeding, the incidence of 40% congenital hydrocephalus in this litter, concomitantly with no history of teratogenic exposure, the presumptive cause of hydrocephalus in our case is a gene mutation.

Keywords: congenital hydrocephalus, cerebrospinal fluid (CSF), gene mutation, inbreeding.

Hydrocephalus is a neurologic disorder characterised by an excessive accumulation of cerebrospinal fluid (CSF) in the brain ventricles and arachnoid cavity caused by excessive production or inadequate re-absorption into the bloodstream by blockage in the ventricular system. The accumulation of the CSF enlarges the brain leading to increased pressure in the skull, followed by thinning of the cranial bones and compression of the brain including brain atrophy and a variety of symptoms, some of which may be life threatening.

In puppies, this is generally a genetic problem related to the breed. However, in kittens, the most common cause is exposure during the mother's pregnancy to the drug griseofulvin (used to treat ringworm, fungus) or to the feline distemper virus, feline parvovirus, feline coronavirus, but the primary culprits are neural tube defects,

intraventricular hemorrhage, trauma, tumors, and gene mutation caused by inbreeding, more likely in our case. Internal hydrocephalus is the most common brain malformation in dogs, on the other hand the prevalence of congenital hydrocephalus in cats is very low (12).

Most animals with hydrocephalus are puppies or kittens with congenital anomalies that lead to hydrocephalus because they are not born with it, but they are born with the anatomical problems that lead to hydrocephalus. The breeds with a high prevalence in hydrocephalus are those with a anatomically tendency to a domed-head like Chihuahua, Pomeranian, English Bulldog, Shih-Tzu, King Charles Spaniel, Boston Terrier and in cats some evidence had been found in Siamese, Persians and Manx cats (3).

Materials and methods

In our case report we have a one year old female domestic cat at her first litter that has been presented at our veterinary service at the Bioveti Farm Clinic because of an emergency, the impossibility of a normal parturition. The owner relates that she started the birth process the night before and since then she had not successfully expelled any kittens, so we had to intervene to solve this dystocia.

Anamnesis indicated that the cat had not been vaccinated against any disease or any antiparasitic treatments were applied, but the cat has received a treatment to prevent the heat cycle, more likely in the period that she already has been mounted. Regarding the paternal implication, he also did not receive any preventive vaccination or antiparasitic treatment.

A cesarean section was performed to obtain five kittens, as on preliminary examination two of them had enlargement of the head circumference, exophtalmos, hypertelorism, frontal bossing and asymmetry of the bony orbit signs that indicate the presence of hydrocephalus (Fig. 1). One of them presented an enlarged and edematous body characterized by infiltration of subcutaneous and interstitial tissues of the fetus with a clear serosity leading to signs of fetal anasarca. Two of the kittens were normal, with no neurological or visible signs of any disease and viable, but smaller than their littermates (Fig. 2).

The data we obtained from the owner indicate that the kittens are the result of an inbreeding between the sister and the brother. Taking into account the possibility of inbreeding again, an ovariohysterectomy was performed with the owner’s consent (Fig. 4).

Because no history of a teratogenic exposure is known and the fact that the kittens are the result of inbreeding leads to the idea that the presumptive cause of 40% congenital hydrocephalus in this litter is genetic.

General clinical signs

Hydrocephalicus can be diagnosed by measuring the head circumference, and puppies often show a retarded skeletal growth compared to their littermates (Fig.3). There are no references to normal head dimensions in dogs and cats, and pure morphological diagnosis can be a challenge in some individuals. However, the most reliable indicators in cats are the presence of frontal bossing, hypertelorism, and asymmetry of the bony orbit (12).

Fig. 1. Fetuses with anasarca (Ist) and hydrocephalus (IInd, IIIrd)

Fig. 2. Viable fetuses from the same litter

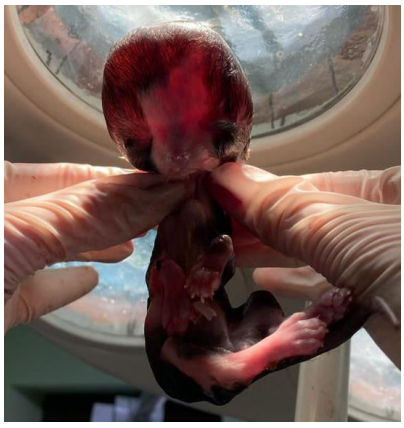

Fig. 3. Evidence of the presence of liquid into the skull

Fig. 4. Mother alongside with the abnormal kittens

If the kittens had lived, some of the clinical signs that would have been present are neurological signs, impaired vision, and ventrolateral strabismus, among others.

Neurological Signs

Depending on the time of onset and the progression of intraventricular pressure, there is significant variation in clinical presentation and severity of clinical signs. Affected kittens may not have any obvious neurological symptoms, especially when they are very young. Clinical signs may be rather nonspecific, including poor appetite, decreased muscle tone, and respiratory difficulties (11, 12).

Impaired Vision

Kittens are born with sealed eyes to protect the immature optical system and are therefore functionally blind. When they start to open their eyes at about 2 weeks of age, the optic system is still not fully developed. It takes around 2 weeks for their eyelids to open and to take on response to their environment. It will take several more weeks before their visual system matures and their eyesight begins to approach normal conditions. Kittens often appear hesitant when walking or may bump into objects and misjudge heights. This may only be noticed when furniture and other objects are moved, because cats have a tremendous ability to adapt to impaired vision (16).

Ventrolateral Strabismus

Bilateral ventrolateral strabismus ("sun-setting sign") is a common symptom of hydrocephalic strabismus. Siamese, Birman, and Himalayan cats frequently demonstrate congenital abnormalities of the visual pathways leading to strabismus (isotropic) and pendular nystagmus (6).

Diagnosis of Internal Hydrocephalus

Imaging data complement the information obtained by morphological phenotype and neurological examination.

Radiography

Radiographic examination of the skull in kittens can only give an indication of pathological growth of the cranium and is generally not helpful in obtaining a definitive diagnosis. Radiographs of the skull alone are usually not helpful for a definitive diagnosis (12).

Ultrasonography

The essential part of the evaluation of neonatal animals with suspected hydrocephalus is to assess ventricular size and to detect or exclude other malformations that influence treatment and prognosis. Ultrasonography is a safe and minimally invasive modality for imaging the ventricular system. A transcranial approach can be performed in conscious animals, which is crucial for the examination of neonatal patients. An open fontanel provides an acoustic window for ultrasound waves and allows visualization of the lateral and third ventricles even in skeletally mature patients. With gentle handling and bending of the head, the caudal (posterior) cranial fossa, brainstem, and cerebellum can be examined through the foramen magnum (10).

Doppler Ultrasonography

Doppler ultrasound is used to measure blood flow from the basilar artery and to determine the resistive indices of the cerebral arterial (10).

Differential diagnoses

The diagnosis should never be based of MRI, on the findings only but also on patient signalment, history, and exclusion of other parallel conditions in the patient. Ventriculomegaly is often mistakenly diagnosed as hydrocephalus, and the actual underlying cause of the presenting clinical signs is missed (12).

Results and discussions

It is important to recognize that hydrocephalus is not a specific disease, but rather a multifactorial disorder with a variety of physiopathology mechanisms: primary genetic abnormalities that may occur individually and secondary injury mechanisms that are expressed primarily because of a pathology of the CSF (7, 14).

The CSF circulates through the ventricular system into the subarachnoid space, where it is absorbed by the arachnoid villi. Obstruction to CSF flow, which can occur anywhere along the pathway from its formation to the site of absorption in the cranial and spinal arachnoid villi, causes active distension of the ventricular system (14). Cerebrospinal fluid is produced at a constant rate by the choroid plexus of the lateral, third and fourth ventricles at a rate of about 0.017 mL/min in the cat by two distinct processes. The main congenital mechanisms involve aqueductal stenosis or obstruction, ependymal denudation, and alterations in the subcommissural organ (SCO). After its production, the ventricular CSF flows through a series of narrowings from one compartment to the next, beginning with the lateral ventricles, through the interventricular foramina to the third ventricle. From there, it flows through the mesencephalic aqueduct to the fourth ventricle. CSF circulates through the ventricular system into the subarachnoid space, where it is absorbed to its main site by the arachnoid villi located in the cerebral veins and venous sinuses by a passive process (7).

Hydrocephalus can be classified as congenital or acquired. Based on the location of the accumulation of CSF, it can be classified into various types such as internal hydrocephalus, where the accumulation of CSF is within the ventricular system, external, where the accumulation is located within the subarachnoid space. Communicating hydrocephalus results usually from an obstruction beyond the fourth ventricle that communicates with the subarachnoid space. An obstructive (non-communicating) hydrocephalus is ventricular dilation result from a lesion that causes obstruction of CSF flow before entering the subarachnoid space. When there is an increase in the volume of the CSF that occupies the space formerly used by the lost parenchyma, it is known as compensatory hydrocephalus (3).

Another leading cause of hydrocephalus in cats that can be possible in our case because the cat was not vaccinated is the infection with the Feline Coronavirus, which creates a fatal immune-mediated disease known as feline infectious peritonitis (FIP), the virus replicates in enterocytes and can pass the enteric border into the blood. Specific to this virus are the mutations that can happen, mutations that lead to the ability to invade and replicate in macrophages causing violent multisystemic

inflammatory reaction. This reaction can have two forms, granulomatous or effusive. In the granulomatous form there is no inflammatory exudation into the body cavities, which is a classical clinical sign in the effusive form. The granulomatous form involves the central nervous system (CNS). The virus causes a cellular inflammatory reaction with inflammatory cells that form small granulomas around CNS arteries and venules. This periventricular vasculitis leads to the exudation of a fluid rich in cells and reactive proteins and periventricular reactive astrocytosis (13, 17).

The cause of death of the kittens was probably brain impairment caused by excessive distension of the ventricles leading to high intracranial pressure; furthermore, ventriculomegaly present in the fetal phase may cause extensive brain damage and death of neurons that are responsible for metabolism and circulatory functions; therefore, this predicts a worse prognosis. Understanding the physiopathological mechanisms the presented clinical signs were incompatible with life (9).

Regardless of the mechanisms of the injury, the duration and magnitude of ventriculomegaly leads to different levels of pathology. The first targetted structures that are affected are the periventricular axons, myelin, and microvessels and secondary there are changes in neurons reflecting responses to axonal disconnection, diminishes cerebral blood flow, ischemia, and altered metabolism. The main roles in acute and chronic injury are gliolisis and neuroinflamation (7).

If the hydrocephalus conception products were viable, there were some treatment options, such as medication with diuretics and glucocorticoides and surgically by placing a ventriculoperitoneal shunt with the main goal of reducing the amount of extra fluid to minimize brain damage (1).

Medical management of hydrocephalus is directed at decreasing the production of CSF. This treatment may be performed in cases where surgery is not an option or in an attempt to stabilize ventricular size and clinical signs until a ventriculoperitoneal shunt can be placed (8).

Antiepileptic drugs may also be needed if patients are experiencing seizures. Medical therapy may stabilize or improve signs in the short-term, but often it is not successful in the long-term. Diuretics are used to reduce the production of CSF. Acetazolamide, a carbonic anhydrase inhibitor, has been shown to decrease the production of CSF by the choroid plexus in both dogs and cats. (5, 15)

Glucocorticoids are commonly used in patients with hydrocephalus, although little published information is available on their efficacy in these patients. Tipically, an anti-inflammatory dose is used initially; once clinical signs have improved, the medication is reduced to the lowest dose that still controls clinical signs. Hyperosmolar therapy with agents such as mannitol is occasionally used when there is evidence of intracranial hypertension (8). Furosemide has also been shown to decrease CSF production and intracranial pressure (4).

Conclusions

Hydrocephalus is a multifactorial disorder with a genetic cause, an environmental cause or both.

In cats, the genes responsible for those mutations are not known for sure, compared to other species.

Inbreeding, determines a phenotypic expression much more evident of the mutant genes, in our case 40% of the litter.

The literature suggests the existence of some possible treatment with the purpose of maintaining a normal level of cerebrospinal fluid.

Acknowledgement

We would like to express our greetings to Dr. Pall Mihaly and the Bioveti Farm staff who gave us the opportunity to do our practice stage in this clinic and to collect the data for this rare case and also to motivate us to do our research on this condition.

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