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ŞI MEDICINĂ VETERINARĂ A BANATULUI
TIMIŞOARA**

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SKIN TUMORS IN DOGS AND CATS: A REVIEW

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Summary

In domestic animals, skin diseases are often diagnosed in small animal general practice (20-75% of the cases). Due to an uncontrolled proliferation of cells, that are unable to act accordingly to the signals that rule a normal cell behavior (division, growth), tumors develop. Consequently, this will lead to an invasion of healthy organs and tissues. Some of these cells will even metastasize to different areas of the body. For dogs and cats, tumors can be neoplastic or non-neoplastic. For proliferative lesions that involve an increase in the cutaneous volume, the term skin tumor is used. Considering that the skin is the largest organ, acting as an intermediary between the external environment and the body, it's easy to understand why there is such a high incidence of skin neoplasms (the most diagnosed neoplastic disorder in dogs and cats are skin tumors, approximately 30% of all diagnosed tumors). Tumors of the subcutaneous tissue and the skin represent 33% of tumors in dogs and 25% of tumors in cats, out of all diagnosed tumors. In dogs, the majority are benign (70-80%), while in cats, more than half are malignant (50-65%). Metastatic lesions are not common, and most tumors are primary. When it comes to the incidence of skin tumors, dogs show a lower mean age than cats, in both males and females. The most common skin tumors in dogs are (descending): mast cell tumors, lipomas, and histiocytomas, and in cats are (descending): basal cell tumors, mast cell tumors, and squamous cell carcinomas.

Keywords: tumor, skin, veterinary oncology, dog, cat.

A tumor is an abnormal benign or malignant new growth of tissue that possesses no physiological function and arises from uncontrolled usually rapid cellular proliferation (36).

Cancer is not a simple, or a single disease. The term is used to describe several diseases, the common feature being uncontrolled cellular proliferation and growth. The only requirement for the manifestation of cancer is multicellularity. Animals, humans, and plants suffer from neoplastic diseases, cancer being a major health concern (6, 13, 32).

'Cancer' is an umbrella term: tumor ('swelling') is generically used to describe any mass, regardless of its characteristic as benign or malignant; neoplasia ('new growth') is the correct scientific term that describes the pathological process of abnormal cell growth; cancer refers to malignant tumors/ neoplasm; oncology is the specific study of all the previously mentioned (6, 27).

In 5% of the cases, neoplasms are considered an inherited genetic disorder, while in the other 95% they appear spontaneously and are linked to different

environmental factors, that will produce changes (directly or indirectly) in the cellular genome (7).

Due to an uncontrolled proliferation of cells that are unable to act accordingly to the signals that rule a normal cell behavior (division, growth), tumors develop. Consequently, this will lead to an invasion of healthy organs and tissues. Some of these cells will even metastasize to different areas of the body (10).

In many species, ionizing radiation and thermal injury have been reported to increase the risk of skin cancer. Ultraviolet radiation (UV) has long been known to cause neoplastic transformation and is a major contributor to rising rates of skin cancer in humans. Evidence for the role of UV irradiation in the development of skin tumors in cats and dogs is primarily epidemiologic and supported by case reports on dogs diagnosed with a spectrum of sunlight-induced lesions (37).

The fact is that tumors are more commonly observed in older patients, so, this highlights the idea that, over time, a normal cell transforms into a tumor cell under the synergistic action of a combination of factors (15).

In companion animals, cancer remains the most common disease, reaching 15-30% in dogs and 26% in cats (some studies suggest that one in four cats/ dogs will die because of cancer/ cancer-related disease) (6, 7, 13).

The skin is considered to be the organ that's most affected by diseases, while also being one of the main sites where neoplasms develop. In small animal general practice, 20-75% of the cases are represented by different skin diseases. There is a variety of non-tumoral and tumoral dermatopathies with similar clinical manifestation/macroscopic appearance, which highlights the importance of a correct diagnosis (12, 14, 30).

For proliferative lesions that involve an increase in the cutaneous volume, the term 'skin tumor' is used. Considering that the skin is the largest organ, acting as an intermediary between the external environment and the body, it's easy to understand why there is such a high incidence of skin neoplasms (the most diagnosed neoplastic disorder in dogs and cats are skin tumors, approximately 30% of all diagnosed tumors, dogs being slightly more affected than cats) (6, 12, 15).

Because of the skin's complex nature (epithelial, mesenchymal, neural, neuroectodermal tissues) and the big number of species in the animal world, the classification of tumors in veterinary medicine is controversial. Skin tumors are often the single most common histopathology specimen sent to laboratories for diagnosis, even when it comes to dogs and cats (9, 17, 30).

Tumors will derive from the epithelial cells of the epidermis and adnexa; the melanocytes, dermal stromal cells, subcutaneous adipocytes, or infiltrating hematopoietic cells (11).

Materials and methods

For this review, clinical studies that were conducted on the subject at hand (veterinary oncology - skin tumors, species - dogs and cats), were selected (using

PubMed, Web of Science, Google Academic), as well as other publications of interest, all from the last 10 years.

Results and discussions

The overall incidence of skin tumors in dogs and cats is very difficult to determine because there is an inconsistency in reporting the condition. However, it is considered that tumors of the subcutaneous tissue and the skin represent 33% of tumors in dogs, and 25% of tumors in cats, out of all diagnosed tumors (37).

In dogs, the majority are benign (70-80%), while in cats, more than half are malignant (50-65%). Metastatic lesions are not common, and most tumors are primary (26).

In both cats and dogs, it was noted a higher incidence of skin tumors in older animals. Also, there is a tendency for females to be more affected by neoplasms, in both species (10).

The incidence of different skin tumors in dogs is mast cell tumors (highest), followed by lipomas, histiocytomas, perianal glands adenomas, sebaceous gland hyperplasia/ adenomas, squamous cell carcinomas, melanomas, fibrosarcomas, basal cell tumors, and papillomas (14, 26, 32).

In cats, basal cell tumors are the most frequently encountered skin tumors, followed by mast cell tumors, squamous cell carcinomas, fibrosarcomas, apocrine adenomas, lipomas, hemangiosarcomas, sebaceous adenomas, fibromas, hemangiomas (9, 26, 32).

In all cases in which a tumor is suspected, after the clinical evaluation and the recognition of the mass on a gross level, a biopsy is required (cytology/ histopathology). Lymph nodes should be examined, and any other evaluation that may help to establish the diagnosis and the general state of the patient (respectively the prognosis and the most appropriate treatment option) should be performed - medical imaging (ultrasound, radiography, computer tomography) or different paraclinical examinations. An overview of the animal's health is very useful, even if the tumor itself doesn't seem to bother the patient to a greater extent. A correct diagnosis (ideally confirmed as early as possible) is vital in oncology, as it helps in choosing the right treatment and also maximizes the chances of success when it comes to the outcome.

The present study gives a brief but comprehensive characterization of 6 common types of skin tumors in dogs and cats (basal cell tumor, histiocytoma, lipoma, mast cell tumor, melanoma, and squamous cell carcinoma), underlining the specific aspects for each of them.

Basal Cell Tumor (BCT)

The term 'basal cell tumor' was used as an umbrella term for basal cell carcinoma (BCC), basal cell epithelioma, trichoblastoma, and solid-cystic ductular sweat gland adenomas/ adenocarcinomas. Due to the progress made in oncology

trichoblastomas and solid-cystic ductular sweat glands tumors are no longer in this category. Many times, the origin of all tumors in this category cannot be identified (32, 37).

BCC is the most common among the BCTs that have a low-grade malignancy. It is rare in dogs and it presents as a plaque or as nodules, that are darkly pigmented. Dogs regardless of age can be affected. Until now, clinical studies are unclear about a possible breed predisposition (32).

In cats, BCCs are considered to be the most common skin tumors, but there is a long discussion about their real incidence after the reclassification of BCTs. Older cats are more affected. As it is seen in dogs, clinical studies could not determine sex or breed predisposition, although long-haired cats may be more prone to this condition. Usually, they are benign. There is a predilection for the head and the neck, but the tumors can occur anywhere in the body. They can resemble melanomas, as they appear pigmented. They are well-circumscribed and can show alopecia or ulceration. Extensive infiltration in the dermis and subcutaneous tissues is often noticed (17, 32).

Histologically, there are 2 variants of BCCs: the infiltrative type and the clear cell type (17).

The treatment is wide surgical excision. Recurrence after surgery was reported. Other treatment options are unfortunately not as well documented, so the data is limited on this subject (37).

Histiocytoma

A canine cutaneous histiocytoma is a benign tumor. It's considered to be up to 14% of all canine skin tumors. The starting point of these tumors is the Langerhans cells, from the epidermis. It's usually encountered in young dogs that are under 3 years old, and a breed predisposition was noticed (Bull Terrier, Boxer, Dachshund, Cocker Spaniel) (6, 11, 18).

They are typically solitary, single or multiple, button-shaped nodules and appear on the cranial aspect of the body. Histiocytomas most often infiltrate the dermis and may manifest epidermotropism with intraepidermal clusters; the infiltrates usually extend to the deep dermis (8, 11, 24).

Even if multiple, tumors often resolve spontaneously in a few months. If Langerhans cells migrate in lymph nodes, lymphadenopathy may be seen (6, 8).

Surgical excision is the preferred treatment method when dealing with this condition. The use of Lomustine (chemotherapy) has been described as successful (4, 6, 11).

Lipoma

Lipomas are slow-growing, benign tumors of adipose tissue. There are 3 morphologic types of lipomas: intermuscular, regular, and infiltrative. Lipomas, especially the regular ones, are often seen in older dogs (17, 32, 35).

Despite the evidence showing a relatively frequent occurrence in dogs, there is very little published evidence on risk factors for lipomas. Advanced age and excessive body weight are risk factors. Females are overrepresented. (23).

Dogs weighing at or above the mean for their breed and sex had 1.97 times the odds of diagnosis with lipomas (23, 34).

The breeds most at risk are Doberman Pinschers, Labrador Retrievers, Miniature Schnauzers, and mixed-breed dogs (29).

They are usually asymptomatic. They occur in the trunk, gluteal region, and proximal limbs. The tumors are well-circumscribed, unencapsulated, and soft. Most of them are freely moveable over the deeper tissue. Only a small percentage are infiltrative (17, 32).

Despite the low danger to the life of the animal, the welfare of the animal may be compromised, due to its characteristic growth and ulcer formation, with associated pain, thus lipomas should not be ignored (5).

For the majority of dogs with non-infiltrating lipomas managed in primary care practice, lipomas are not often debilitating, and surgical removal has low postsurgical complications (25).

Surgical resection is most often curative, although recurrence can be noticed. The infiltrative ones may be harder to excise at the time of the first surgical intervention (32, 35).

Mast Cell Tumor (MCT)

Mast Cell Tumors in dogs

MTCs are the most common skin tumors in dogs, with a highly variable metastatic rate (from 10% to more than 95%). MCTs are not easy to manage and usually can require multimodal therapy (6, 22, 24).

There is not enough evidence to indicate the exact etiology (chronic inflammation was suspected). Older dogs are more affected. Boxers, Boston Terriers, Beagles, American Bull Terriers, Pugs, Dachshunds, Golden Retrievers, and Labrador Retrievers are predisposed to this condition, among others (6).

MCTs can be cutaneous or subcutaneous. Most of them are seen on the trunk, followed by the extremities, and the head/ neck, even though any region of the body can be affected. Visceral or systemic mastocytosis occurs consequently to the dissemination from an aggressive primary cutaneous tumor. The biggest problem with MCTs is that they are great imitators, have a variety of clinical appearances, and can mimic other types of tumors. Also, the gross appearance should not be considered an indicator of the lack of malignancy of the tumor. Erythema and edema are usually seen. In aggressive MCTs, the following can be noted: rapid growth, local infiltration, irritation, inflammation, ulceration, and satellite nodules (1, 21, 22).

Almost half of the dogs have complications resulting from the release of bioactive substances from MCTs, with the following being the frequent ones: erythema, local hemorrhage, poor wound healing, and gastrointestinal ulceration (1, 6, 21).

If wide surgical excision is possible, then surgery should be the first option. If not, chemotherapy (first line: Vinblastine and Prednisolone, second line: Lomustine; the good response was obtained with the combination of Vinblastine, Cyclophosphamide, and Prednisolone), multimodality therapy or palliative therapy should be considered (1, 6, 24).

It's mandatory to treat patients with MCTs for the systemic signs associated with the condition. The more advanced the disease, the poorer the prognostic. Relapses are often reported (6).

Mast Cell Tumors in cats

MCTs are considered to be up to 15% of all skin tumors and are most of the time benign. The average age of the animals for this tumor is 9-11 years and Siamese cats are predisposed (6, 16).

In many cases, MCTs present as firm alopecic nodules, that can be solitary or multiple, pale or tan when it comes to color. It usually occurs on the tail, head, and limbs. In cats, cutaneous metastases from visceral MCTs can develop. When characterizing the tumor from a histological point of view, there are 2 forms: compact or diffuse; the compact ones are more common, minimally invasive, and do not metastasize (6, 16, 22, 28).

The treatment of choice is surgical excision (if the tumor is diffuse, the margins will be wider). Vinblastine, Chlorambucil, and Lomustine have been reported as being used, along with Prednisolone. The use of radiotherapy is not well documented (6).

Melanoma

Melanomas are malignant neoplasms originating from melanocytes. Cutaneous melanoma is more often diagnosed in dogs than in cats. In cats, more than half of them are malignant, while in dogs 2/3 are benign (although malignant transformation may occur). Most of the affected animals are older, and while this condition is not considered to be UV-linked, animals with black/grey coats or pigmented skin are at risk (6, 20).

In cats, cutaneous melanoma is rare, with intraocular melanoma being more frequently observed (iris melanoma is the most common intraocular tumor in cats) (33).

In dogs, melanomas are most commonly observed in Scottish terriers, Poodles, Golden retrievers, Dachshunds, Cocker spaniels, Chow Chows, Gordon setters, and Anatolian Sheepdog breeds, although the true incidence in individual breeds of dogs is poorly established (31).

The tumors can occur on different parts of the body, but there is a specific distribution for the benign tumors (head for cats, head/trunk for dogs) and the malignant ones (head/trunk/tail for cats and lips/eyelids/limbs for dogs). When benign, melanomas present as well-circumscribed, pigmented, alopecic nodules (smooth, sessile, pedunculated, plaque-like, papillated). Infiltration and ulceration are more often noticed when the tumor is malignant (6, 20).

The anatomic site is high, though not completely, predictive of behavior including local invasiveness and metastatic propensity. Most melanomas at dermal sites are benign and metastasis or recurrence after adequate excision is rare. In contrast, melanomas of the oral cavity, digits, and mucocutaneous junctions are associated with a poor prognosis due to a high degree of local invasiveness and metastatic propensity (2, 20).

Histological appearance does not always correlate well with biological behavior (2).

Usually, the treatment consists of surgical excision, but the overall cure rate is not high (around 35%). Radiotherapy can also be beneficial. Chemotherapy has no proven effects (6).

Squamous Cell Carcinoma

Squamous cell carcinoma is the second commonest malignant skin tumor in dogs and the commonest in cats. Squamous epithelium forms most of the skin, lines the oral cavity and esophagus, and forms the nail beds and foot pads (19).

The incidence is largely dependent on different geographical factors, that are related to UV exposure. Animals with white hair coats for cats and white or short hair for dogs are at risk. In white cats, the chances of developing squamous cell carcinoma are 13 times higher. This neoplasm has also been reported after thermal injuries and in cases with chronic inflammation. In both dogs and cats, older animals are more prone to this disease (6, 17).

Affected regions for cats are the nasal planum, pinnae, and eyelids and for dogs: the ventral abdomen, flanks, and medial aspect of the thigh. The neoplasm can be plaque-like, or a crateriform, ulcerated, fungiform mass. Crusting is also common. In dogs, when the nasal planum is affected, the lesions are ulcerated and the tumor is aggressive and invasive, with nodal metastases being present in many cases. Multifocal squamous cell carcinoma may also occur (3, 6).

Staging is particularly important for nasal planum tumors as the success of treatment is directly impacted by the stage of disease (3,19).

Radiation therapy and photodynamic therapy can be used in the early stages of the disease (but recurrence is common), as the advanced one requires surgical management. Systemic chemotherapy is not particularly successful and it’s not usually recommended. A cryosurgery is also an option for superficial tumors (3, 6, 19).

The prognosis is variable and it’s also dependent on the invasiveness of the tumor, as well as the efficacy of the treatment (6).

Conclusions

Skin tumors are the most commonly diagnosed type of tumors in dogs and cats because they are easily seen and identified by the owners, usually causing concern, so the animals are brought to the veterinarians for a check-up.

It's important to do a full and comprehensive evaluation of the patient. Clinical and paraclinical examination, medical imaging, biopsy sampling, and cytology/histopathology are mandatory when dealing with an alleged oncology patient, in order to make a correct diagnosis and to choose the most appropriate treatment protocol.

The treatment options for skin tumors are various and depend on the type of the tumor: surgical excision (usually the recommended treatment, based on the principles of oncological surgery), radiotherapy, chemotherapy, laser therapy, angiogenic therapy, immunotherapy, and others.

Usually, owners have some information (or misinformation) about tumors in general, so the approach should be made in a compassionate, positive, and knowledgeable manner while being as realistic as possible. The better an owner understands the animal's situation, the better the chances to find an appropriate solution to each specific case. The owner should be well-informed and up-to-date with the treatment and the prognosis.

Skin tumors are very diverse and the therapeutic approach usually has limitations - owner financial constraints or inaccessible equipment/medication. The prime directive should be to ensure the quality of life and the health of the patients, during the whole process.

Cancer is a disease that knows no species boundaries, the veterinarian finds himself playing a key role in the comparative oncologic investigation, the goal of this being to cure, or at least to transform cancer from an acute life-threatening disease into a manageable chronic condition, regardless of the species. The dog is considered to be the best study model, because they live close to humans, in the same environmental factors that can cause cancer, and they share similarities when it comes to the histology and biology of this disease (numerous cancer types, like osteosarcomas, melanomas, mammary carcinomas, non-Hodgkin lymphomas, are similar in dogs and humans).

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USING THE SEROTONIN AND CORTIZOL VALUES AS A TOOL FOR WELL-BEING ASSESSMENT IN DOGS

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Summary

Neurotransmitters like serotonin (5-HT) and steroid hormones (glucocorticoids) have a central role in animal behavior. Serotonin is involved in neuronal excitability regulation and affects mood, cognition and behavior. A large number of psychopharmacological data implicate serotonin in animal models of depression and anxiety. The increased hypothalamic-pituitary-adrenal (HPA) axis response is one of the most reported physiological responses to animal stress. Cortisol, which is secreted by the cortex of the adrenal gland, is the main glucocorticoid hormone in dogs. Cortisol plays a primary role in the acquisition, deposition and mobilization of energy and, at high levels, it modulates changes associated with stress. In dogs, increased plasma cortisol levels can indicate acute stress from sudden fear-inducing stimuli. Through this paper, an attempt was made to highlight the feeling of well-being in dogs, expressed by the values of serotonin and cortisol in the blood flow, corelated to positive and negative stimuli. Serotonin, which is also called the "hormone of happiness" was the reason why we wanted to determine its variations depending on the positive or negative stimulus applied to a dog. In this study, 5 dogs were included, which were exposed to positive and negative stimuli. The blood samples were collected immediately after the application of each stimulus, from the cephalic vein, in vacutainers without anticoagulant. The analysis of blood samples and the determination of serotonin and cortisol was carried out at a private laboratory. After the application of a positive stimulus, the mean values of serotonin were 341.43 ug/l, cortisol 2.0 ug/ml, significantly lower ($p \leq 0.01$) with 30.81 ug/ml and 0.6 ug/ml ($p \leq 0.05$) compared to the application of the negative stimulus serotonin when the serotonin and cortisol values increased to 372.24 ug/l, as well as cortisol, at 2.6 ug/l. After the dogs calmed down, serotonin and cortisol values were 399.87 ug/l and 2.1 ug/dL) compared to the state induced by the negative stimulus. Between the application of the positive stimulus and the induction of the calm state following the negative stimulus, the significant difference of 58.44 ug/ml ($p \leq 0.01$) was recorded only for serotonin, cortisol having appropriate and insignificant values ($p \geq 0.05$)
Keywords: cortisol, serotonin, dog, behavior.

Within the existing literature for canines, both plasma cortisol and serum serotonin have received attention for possible correlations with aggressive behavior traits in dogs. Three studies specifically investigated the correlation between low serum serotonin concentrations and owner-reported histories of aggression toward people or dogs (15, 16, 17). While all of these studies concluded that aggressive dogs typically have lower serum serotonin concentrations than dogs with no history of problematic aggression, there was significant overlap of the mean serum concentrations of the aggressive (209.6–318.6 ng/mL) and nonaggressive sample groups (282.5–852.77 ng/mL) between studies. As such, existing data provide

inconclusive evidence that serum serotonin measures are useful indicators of aggressive tendencies in an applied environment, and a reliable reference range would need to be determined before serum serotonin could be used to help identify pet dogs with aggressive tendencies.

Neurotransmitters like serotonin (5-HT) and steroid hormones (glucocorticoids) have a central role in animal behavior. Serotonin is involved in neuronal excitability regulation and affects mood, cognition and behavior (1). A large number of psychopharmacological data implicate 5-HT in animal models of depression and anxiety (2). Serotonin is suggested to be anything from a fine modulator during stress to a neurochemical grossly affected by stressors, possibly as a reflection of the activity/arousal level of the individual (3, 8, 9). A decrease in 5-HT levels may be a signal of chronic stress since sustained stress leads to the diminution of 5-HT turnover (4, 11, 12). Serotonin and stress hormone disturbances in depression are of pathophysiological significance and not merely a consequence of the depressed state or a product of stress generated by the depressed state (7, 18, 19).

The increased hypothalamic-pituitary-adrenal (HPA) axis response is one of the most reported physiological responses to animal stress (10, 14). Cortisol, which is secreted by the cortex of the adrenal gland, is the main glucocorticoid hormone in dogs (5, 6). Cortisol plays a primary role in the acquisition, deposition and mobilization of energy and, at high levels, it modulates changes associated with stress (12). In dogs, increased plasma cortisol levels can indicate acute stress from sudden fear-inducing stimuli (13).

Intraspecific social exposure sessions could be useful in modulating undesirable behaviors in pet dogs (18) as well as increase the social behavior and working ability in military dogs (19). In previous studies, cortisol appeared to be independent of behaviors and signals indicative of play, stress, agonism and mounting (17, 18, 20). Canine marking behavior has been evaluated in relation to cortisol (13). Given that there is a weak relationship between cortisol and marking frequency, it is possible that there is an association between neurotransmitters such as serotonin and marking frequency.

Materials and methods

In ethology, it is more than in any other form of study, it is necessary to prove a change in the behavior of an individual, through biochemical determinations, which can then be compared with each other and then with the physiological values.

Through this paper, an attempt was made to highlight the feeling of well-being in dogs, expressed by the value of serotonin in the blood flow, related to positive and negative stimuli. It was desired to see if, we can say about dogs that they are happy, such as being around, in the presence of the owner.

Serotonin, which is also called the "hormone of happiness" is the reason why we chose to determine its variations after exposure to a positive or a negative stimulus.

Each subject had a cannula fitted in the cephalic vein, and blood samples were taken after the administration of a positive stimulus—the owner petting the dog for 10 minutes (Fig. 1). After ten minutes, the dog was put in an uncomfortable situation, including being irritated by an unknown person, coming into contact with another dog, playing rough with the owner, and being in situations where he was not in the same level of wellbeing as when the owner was petting him (Fig. 2). In the next 10 minutes, the dog is allowed to calm down and another blood sample was collected.

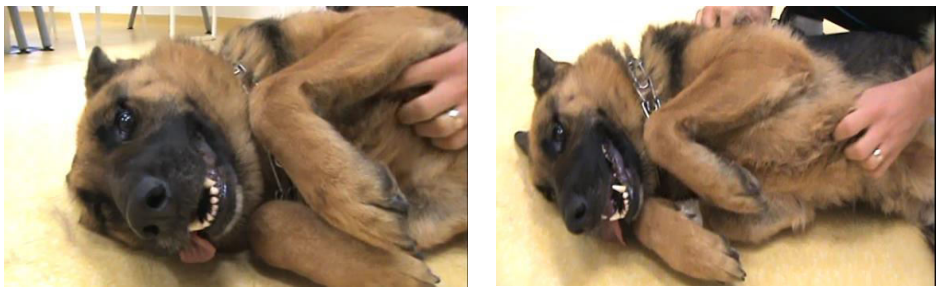

Fig. 1. Positive stimulation

Fig. 2. Negative stimulation with the owner

After the end of the experiment, the blood samples were taken within an hour to the Bioclinica medical laboratory. Serotonin was determined from the serum isolated from the sample, using the HPLC (High Performance Liquid Chromatography) method. The determination of cortisol was made from the serum, by the Siemens Chemiluminescent method.

Results and discussions

After the application of a positive stimulus, the mean values of serotonin (Fig. 3) were 341.43 ug/l, cortisol 2.0 ug/ml (Fig. 4), significantly lower ($p \leq 0.01$) with

30.81 ug/ml and 0.6 ug/ml (p≤0.05) compared to the application of the negative stimulus serotonin when the serotonin and cortisol values increased to 372.24 ug/l, as well as cortisol, at 2.6 ug/l. After the dogs calmed down, serotonin and cortisol values were 399.87 ug/l and 2.1 ug/dL.) compared to the state induced by the negative stimulus.

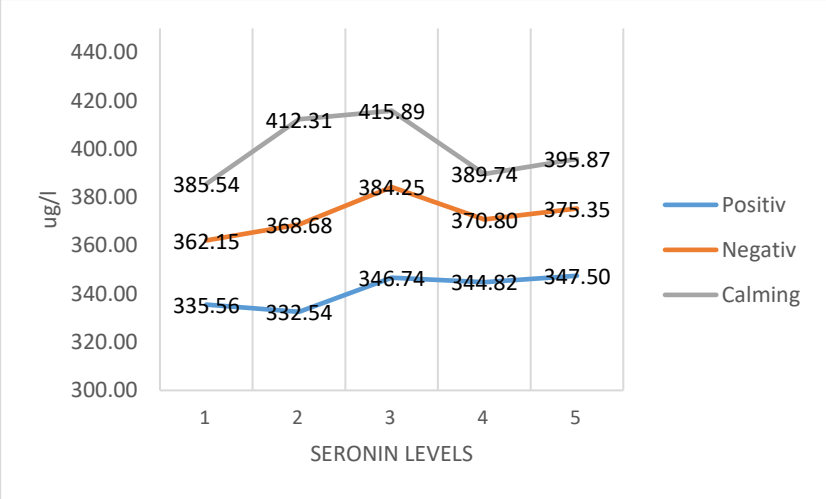

Fig. 3. Serotonin levels

Between the application of the positive stimulus and the induction of the calm state following the negative stimulus, the significant difference of 58.44 ug/ml (p≤0.01) was recorded only for serotonin, cortisol having appropriate and insignificant values (p≥0.05).

Associations between plasma cortisol and behavioral traits are less straight forward than those for serum serotonin, as circulating cortisol is used as an indicator of acute stress in dogs. Even so, several associations have been identified between increased plasma cortisol concentrations and behavioral tendencies, including a history of aggression toward people (13), high scores on “emotionality” and fear-related behavioral measures (9), general behavior problems in puppies adopted from shelters, as reported by owners six months after adoption (9), and fearful behaviors in dogs newly admitted to shelters, during interactions with unfamiliar humans (18). Together, these studies indicate a possible association between increased plasma cortisol concentration when sampled in novel or stressful environments, and fearful or aggressive behavioral tendencies in domestic dogs.

In a previous study conducted by Handlin et al. (8), there were several significant positive correlations between the frequency of activating touch (scratching and patting) and the dogs’ cortisol levels at start of the experiment but also during

the remaining part of the experiment; that is, the higher the dogs' cortisol levels were at start of interaction the more activating touch they received and the higher their cortisol levels became. Besides for activating touch there were no significant relationships between the other forms of touch studied and the dogs' cortisol levels.

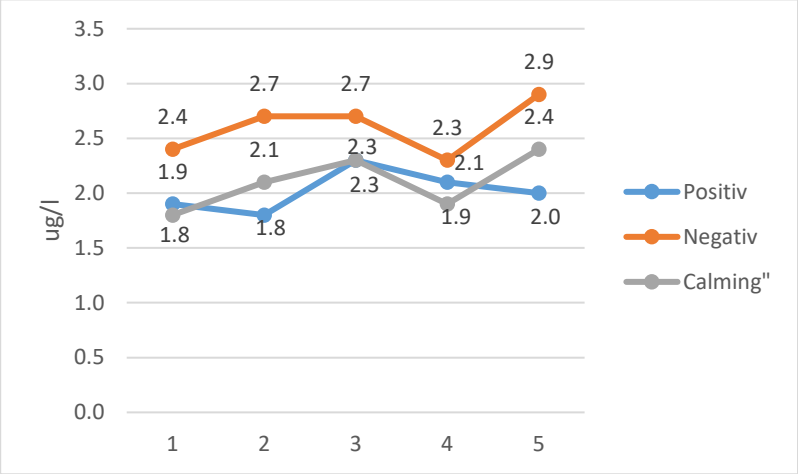

Fig. 4. Cortisol levels

The dogs' cortisol levels correlated positively and significantly with the amount of activating touch they received from their owners. In everyday-life interactions between dogs and owners, the activating type of touch is probably used more frequently during play. On the other hand, the stroking type of touch is probably used more frequently during calm interaction between owners and their dogs and hence it might have a more calming effect on the dogs. The activating touch applied to the dogs in this study might therefore have triggered an expectation of play in the dogs.

Conclusions

After the application of a positive stimulus, the mean values of serotonin were 341.43 ug/l, cortisol 2.0 ug/ml.

At the moment when negative stimuli was applied, the level of serotonin increased significantly with 9.02 % and cortisol with 30%, which denotes a strong emotion.

The cortisol values decreased significantly after the dogs calmed down, by 19.23 %, but serotonin remained higher with 7.25%.

Circulating cortisol and serotonin are poor candidates for use in applied behavioral assessments for mixed-breed pet dogs of varying ages.

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ASSESSING THE BEHAVIOUR OF SHELTER DOGS TO DIFFERENT STIMULI

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Summary

Assessing the behavior of dogs in shelters plays an important role in the adoption process. The literature describes a series of tests, designed to select dogs that can be adopted by those who previously need behavioral therapy. In this study, carried out in a dog shelter in Timisoara, a behavioral test was applied in which the dogs were exposed to natural/artificial stimuli. By natural stimuli we mean the reaction of dogs to contact with the examiner, in the socialization test, and the artificial stimulus was represented by a doll, which simulates contact with a child, in the situation when the dog will be adopted. From 40 dogs subjected to this experiment, the majority, 35, responded in a favorable way to both the natural and the artificial stimulus: 20 to human contact and 15 to the doll. This favorable response is quantified by several criteria: the position of the ears, of the tail, approach, jump on the evaluator, on the doll, smell, lick, play, wag the tail, roll on back. The negative reactions were expressed by 5 dogs toward human and doll, such as: growling, barking, running into a corner of the room, tremble, show teeth, ears back, piloerect, lip lick, biting the doll. Through this experiment we found that dogs in shelters have a constant need to socialize with humans, so even in the presence of a doll, they show positive behaviors.

Keywords: doll, human, dog, behavior, shelter.

Behavior evaluations play a critical role for shelter and rescue dogs, and are used to identify behavior tendencies in order to rehome an animal into an appropriate home (1, 2, 5, 8, 12). Globally, millions of dogs enter shelters each year (7, 9, 10, 13), and in order to be considered available for adoption, they must go through a screening that includes at least two tests: temperamental and behavioral. Dogs that do not meet the requirements (skills) of adoption are euthanized (3, 18, 21, 25). Subsequent research has highlighted the shortcomings of these tests (15, 22), materialized by incomplete standardization as well as poor methodology followed by a scientific validation in the early stages (14, 16, 17, 19).

These observations are a major problem because, based on the results obtained, each individual decides the future of the dogs tested, which has only two possible directions: adoption or euthanasia (4, 6, 11, 23, 26).

Internationally, there are several assessment tests that have been standardized and validated over time, and the most widely used are The Assess-A-Pet program and the Safety Assessment for Evaluating Rehoming test (SAFER).

Assess-A-Pet is an assessment test created by Sternberg (23), who is considered an expert in assessing behavior in dogs. The test lasts 15 minutes: it starts with observing the dog's behavior in an unknown space, continues with the evaluation of the degree of socialization, following the way we play, excitability, guarding resources, behavior towards other animals and using an artificial hand to observe the dog's behavior when trying to take the bowl of food.

Sternberg (23) recommends that dogs can be housed in shelters for at least four days, the time required to perform the tests, which requires at least two people specialized in this purpose.

Safety Assessment for Evaluating Rehoming (SAFER) / Meet Your Match (MYMP) was developed by Weiss (26) and divided into SAFER test and Meet Your Match program. The first part consists of six subtests, which aim to assess the aggressiveness of the dog (6 minutes), as well as the variant proposed by Sternberg (23), the artificial hand is used. After the dog passes this assessment, it is subjected to the second stage, MYMP in which all the individual needs (shortcomings) are identified and according to these we are looking for compatible people who can adopt them (26).

Mornement et al. (18), conducted an evaluation test (BARK), which they standardized and implemented in a shelter, which determined the degree of confidence and predictability (Development of the behavioral assessment for re-homingK9's (BARK) protocol) on dogs. The BARK protocol consists of 12 subtests, through which it is desired to check as many situations as possible, which are possible to be encountered in the daily life of a dog, from a family (18).

This test is based on a study by Mornement et al. (18) who looked at five behavioral traits: anxiety, obedience, fear, socialization, and activity. These traits were evaluated and noted based on the results obtained from the research of several experts in dog behavior.

Materials and methods

The literature describes a series of tests, designed to select dogs that can be adopted by those who previously need behavioral therapy. In this study, carried out in a dog shelter in Timisoara, a behavioral test was applied in which the dogs were exposed to natural/artificial stimuli. By natural stimuli we mean the reaction of dogs to contact with the examiner, in the socialization test, and the artificial stimulus was represented by a doll, which simulates contact with a child, in the situation when the dog will be adopted (Fig. 1, Fig. 2). The evaluator used a large doll, with which he approached the dog and followed the behavior of the individual. The interaction with a doll consisted of introducing, in the room where the dog was already, a doll the size of a two-year-old child (90 cm), with the aim of observing the possible aggressive behaviors that the dog could display towards a child from a future family.

Dogs can show positive behaviors towards this, or on the contrary, become aggressive.

Fig. 1. First contact of the dog with a doll

Fig. 2. Positive reaction toward doll

Results and discussions

From 40 dogs subjected to this experiment, the majority, 35, responded in a favorable way to both the natural and the artificial stimulus: 20 to human contact and 15 to the doll (Fig. 3). This favorable response is quantified by several criteria: the position of the ears, of the tail, approach, jump on the evaluator, on the doll, smell, lick, play, wag the tail, roll on back.

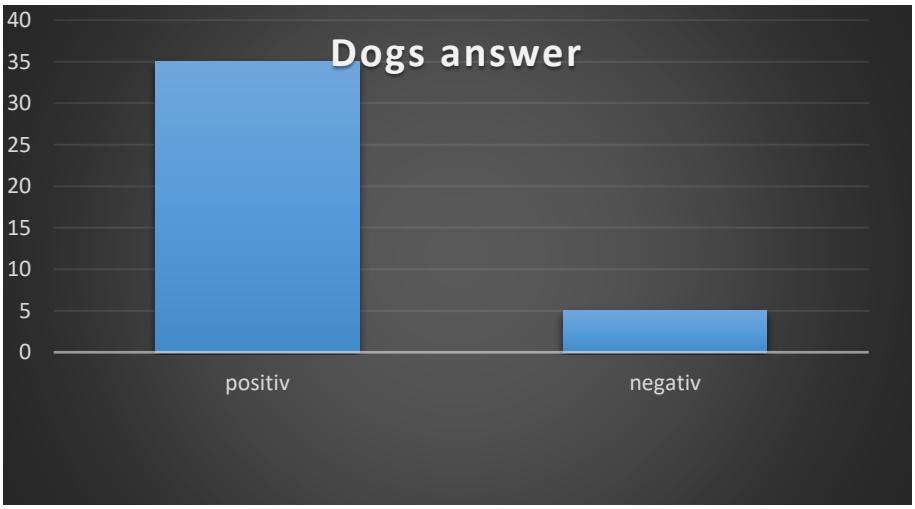

Fig. 3. Dogs' reaction: positive/ negative.

The dogs that showed a positive reaction approached the mannequin, smelled it, expressed playful behavior towards it, adopted a characteristic play position, had a lively look, erect ears, but without showing muscle tension. As a common observation in this section, it was the finding that all the dogs, upon seeing the doll, first smelled it in the mouth region and looked into its eyes (Fig. 4).

The negative reactions were expressed by 5 dogs toward human and doll, such as: growling, barking, running into a corner of the room, tremble, show teeth, ears back, piloerect, lip lick, biting the doll. Dogs that expressed negative behaviors expressed as follows: they smelled the doll, they became tense, moved away from it, without returning, if the mannequin was brought near, the dogs retreated, snarling, barking threateningly at the mannequin, as soon as they entered the test room, as a result they were considered a danger to children.

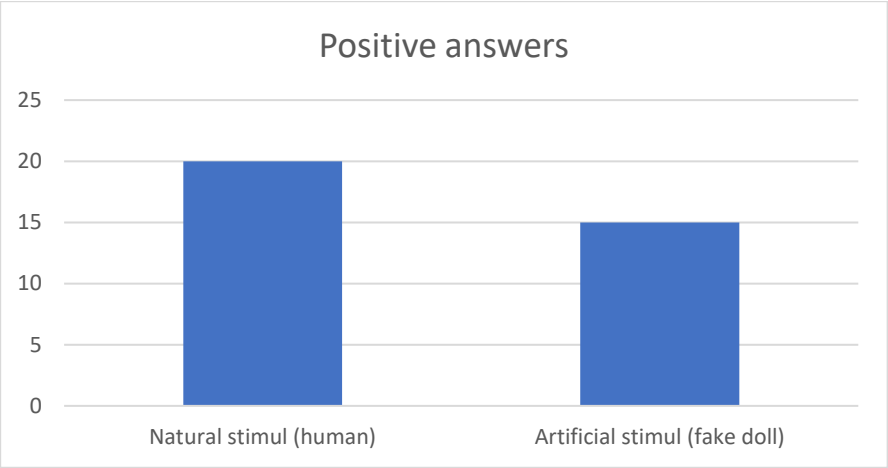

Fig. 4. Dogs responses to fake/real stimuli

In a previous study, conducted by D'Arpino et al. (7), found that the fake dog elicited more aggressive reactions than the real dog although the difference was not statistically significant. One possible explanation is that the fake dog was walked directly toward the test dog with its head up, looking forward, which may have elicited aggressive behaviors, as opposed to the real dog which may have avoided any aggressive signaling toward the test dog. The tendency to display more aggressive behavior toward the fake dog was also noted by Collins et al. (6). In that study, the authors observed pit bull puppies displaying more shaking, holding, and biting behaviors toward the fake puppy than toward littermates or playmates.

Another explanation for the greater frequency and severity of aggressive behavior displayed toward the fake dog could be that the stimulus dog's behavior acted to decrease an aggressive response in the test dog. This idea is supported by previous research showing that it is possible for stimulus dog behavior to influence an aggressive response in tested dogs (10). Netto (20) developed a dog evolution test in which they presented various stimuli that are known to elicit aggression in dogs, including other dogs. To perform a predictability analysis of the test, they re-tested 37 dogs from the original sample. They found that the subtests in which dogs were used as stimuli had the lowest predictability. Since the study staff prevented the stimulus dogs from being "defeated" by the tested dogs, male stimulus dogs reacted more aggressively and the female stimulus dog behaved less aggressively, as the test continued. The authors suggest that inconsistent behavior on the part of the stimulus dogs could have had an impact on tested dogs, resulting in lower predictability rates of dog-to-dog subtest. Moreover, for future studies, the authors

suggest monitoring stimulus dogs closely and replacing them with another stimulus dog if they become too aggressive (20).

Conclusions

In conclusion, the doll elicited a variety of different reactions in tested dogs and seems to be a useful device to evaluate friendly behavior toward humans/ child. However, the aggressive trait showed only a slight degree of agreement between reactions to the real and the fake stimulus.

Through this experiment we found that dogs in shelters have a constant need to socialize with humans, so even in the presence of a doll, they show positive behaviors.

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RESEARCH ON THE IMMUNOGENIC EFFECT OF THREE VACCINES USED IN THE PROPHYLAXIS OF INFECTIOUS BURSAL DISEASE

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Summary

Infectious bursal disease (IBD) is caused by the IBD virus, which has a particular impact on poultry production. In this study, the immunogenicity and the immunosuppression effect of three live-attenuated vaccines (A, B and C), which have different levels of attenuation, were determined in 11 days old Cobb 500 broiler chicks. The vaccines were administered via drinking water. The levels of antibodies (Ab) were determined in the serum of vaccinated chicks on the 7th, 14th, 21st and 28th-day post-vaccination using the enzyme-linked immunosorbent assay (ELISA) method. The results obtained show that all three vaccines induced the synthesis of antibody titres that ensure an adequate level of protection, starting with the interval 7-14 days post-vaccination and later, during the entire monitored period, which corresponds to the interval of maximum susceptibility to the infection. However, the level of antibodies recorded on the 7th-day post-vaccination does not provide protection against natural infection, also vaccine C determined the most significant drop in the Abs concentration on the 7th-day post-vaccination.

Keywords: infectious bursal disease, poultry, vaccine, ELISA.

Infectious bursal disease (IBD) is a viral infection that has a particular impact on poultry production (10) and is of significant importance because it evolves in the vast majority of countries where intensive poultry farming is practised (4, 20).

The annual economic losses caused by this disease are significant and are associated with high morbidity and mortality, which are dependent in particular on the virulence of the infecting strain, the age and breed of the birds, as well as the presence or absence of passive and active immunity (6, 14).

Due to the resistance of the IBD virus in the environment, to the action of usual disinfectants, as well as its persistence in the body of insects, general prophylaxis measures become insufficient, which is why the application of specific prophylaxis is required (7, 8, 19).

The general objective of this study was the evaluation of the immunogenic capacity of three live vaccines with different degrees of attenuation in the prophylaxis of infectious bursal disease and the degree of immunosuppression associated with their administration.

Therefore, the levels of antibodies (Ab) were determined in the serum of vaccinated chicks on the 7th, 14th, 21st and 28th-day post-vaccination using the enzyme-linked immunosorbent assay (ELISA) method.

Materials and methods

In this study, three vaccines were evaluated, two domestically produced, based on attenuated strains of the IBD virus, and one imported, containing an intermediate strain of the IBD virus.

Vaccine A – live, freeze-dried vaccine, containing an attenuated strain of the IBD virus. The vaccine was reconstituted in clean cold water in which powdered milk was previously dissolved (1000 doses of vaccine / 20 litres of water / 100 g of powdered milk). It was administered via drinking water, with each bird receiving a dose of 20 ml.

Vaccine B – live, freeze-dried vaccine containing a highly attenuated strain of the IBD virus. The vaccine was reconstituted in clean cold water containing 0.5% milk powder (1000 doses of vaccine / 20 litres of water / 100 g milk powder) and was administered in the drinking water, with each bird receiving a dose of 20 ml.

Vaccine C – live, freeze-dried vaccine, based on an intermediate strain. Reconstitution of the vaccine involved dissolving it in cold water containing 0.5% milk powder (1000 doses / 10 litres of water / 50 g milk powder). When administered in the drinking water, each bird received a dose of 10 ml of diluted vaccine.

The vaccines were administered under production conditions, in three sheds with approximately 20,000 11-day-old Cobb 500 chicks, raised on the ground. Each shed was considered an experimental lot.

Before the administration, the birds were subjected to a water restriction for 1-2 hours, in the presence of feed provided at discretion. The watering line was opened only after the vaccines were completely consumed.

The research concerned the level of maternal antibodies and also that of post-vaccination antibodies, developed after the administration of the three evaluated vaccines. For this purpose, 20 birds/experimental lots were isolated and identified using a ring placed around the metatarsus.

Blood samples were collected from the above-mentioned birds, in tubes without anticoagulant or coagulation activators, as follows:

- on the day of vaccination, in order to determine the level of maternal anti-IBD antibodies;
- on the 7^{th}, 14^{th}, 21^{st} and 28^{th}-day post-vaccination (corresponding to the age of 18, 25, 32 and 39 days), in order to establish the post-vaccination immune response.

The serum was evaluated by the ELISA technique, using the *Infectious Bursal Disease Antibody Test Kit* (IDEXX Laboratories, Inc., The Netherlands). The antibody titre ≤ 396 was considered negative, and the one with values above 396 was considered positive, assuring a protective value against viral infection.

The statistical analysis aimed at calculating the mean values, standard deviation and coefficient of variability for maternal and post-vaccination anti-IBD antibody titres. Moreover, we compared the averages obtained by applying the one-

way ANOVA test. The differences were considered significant if the *P* value was less than 0.05.

Results and discussions

The immunogenic effect of vaccine A: At the first determination made on the vaccination day, the titre of maternal anti-IBD antibodies had an average value of 1386.9 (Table 1, Fig. 1), which ensures an appropriate level of protection (12). Seven days after the administration of vaccine A, a significant decrease in the level of anti-IBD antibodies was noticed (P<0.001), a fact that can be attributed mainly to the interference of the viral antigen in the vaccine composition. This phenomenon was also observed by other researchers who tested the immunoprophylactic effect of various attenuated and intermediate anti-BIA vaccines (1).

Table 1

The titer of maternal and post-vaccination antibodies in birds vaccinated with vaccine A

No.	Day 11 (vaccination day)	Day 18 (7 days post-vaccination)	Day 25 (14 days post-vaccination)	Day 32 (21 days post-vaccination)	Day 39 (28 days post-vaccination)
1	1287	361	1201	2251	3860
2	1145	230	962	2475	3389
3	1515	206	1294	2206	4017
4	1268	157	1351	2411	3497
5	1409	233	981	2677	4494
6	1132	129	1159	2637	3721
7	1289	182	1232	2347	4015
8	1354	119	1094	2008	3935
9	1399	145	1365	2149	4009
10	1132	160	1238	2390	4466
11	1539	132	1072	2406	3883
12	1401	146	1290	2644	3951
13	1227	105	1194	2623	3724
14	1681	214	1348	2156	3901
15	1432	138	1366	2572	4062
16	1478	169	1072	2169	3571
17	1467	161	1095	2269	3877
18	1681	231	1256	2533	3742
19	1502	193	1286	2581	3318
20	1400	225	1004	2428	3592
X	**1386.9**	**181.8**	**1193**	**2396.6**	**3851.2**
σ	**160.97**	**48.23**	**131.43**	**197.08**	**303.50**
CV%	**11.61**	**26.52**	**11.01**	**8.22**	**7.88**

Legend: X = mean; σ = standard deviation, CV% = coefficient of variability

Beginning with the 14th day, the post-vaccination (p.v.) antibody titre is starting to increase, with statistically significant differences (P<0.001) between the average values obtained at the last three evaluations (days 14, 21 and 28 post-vaccination).

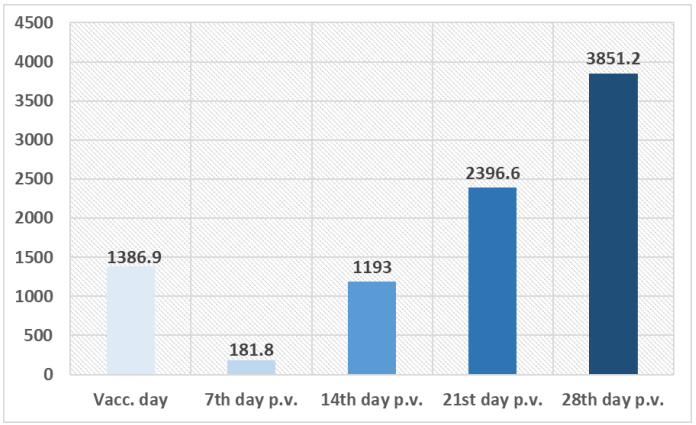


Fig. 1. Graphic representation of maternal and post-vaccination antibody titre in Cobb 500 chicks vaccinated with Vaccine A (mean values)

The most significant increase in the level of antibodies, from one determination to another, is reported on the 14th day after the vaccination, the corresponding average value being 6.56 times higher than that recorded on the seventh-day post-vaccination. On days 21 and 28 post-vaccination, the antibody titre values were almost double, compared to the previous determination.

Regarding the coefficients of variation, the values calculated for days 14, 21 and 28 post-vaccination, demonstrate the homogeneous effect of vaccine A on the studied group. Also, the level of post-vaccination antibodies is adequate to ensure protection for the period of maximum susceptibility to the development of the disease, namely the age of 3-6 weeks (1, 9, 14).

The values obtained are similar to other reports in the specialized literature regarding the immunoprophylactic effects of the BIAVAC vaccine produced by Pasteur Institute, Bucharest, which contains an attenuated strain of IBD or other attenuated vaccine strains (16).

The immunoprophylactic effect of the B vaccine: The titre of anti-IBD antibodies showed a significant reduction (P<0.001) seven days after the administration of vaccine B, compared to the level of maternal antibodies recorded on the day of vaccination (Table 2, Fig. 2).

Between the 7th day and 14th day post-vaccination, the level of anti-IBD antibodies shows a spectacular, statistically significant increase (P < 0.001), the

determination performed on the 14th day providing results approximately ten times higher than the values obtained on the 7th day (2115.4 compared to 229.4). From this moment, although the increase in the level of antibodies is present and statistically significant (P<0.05), it is no longer so obvious because the average post-vaccination antibody titre obtained on the 28th-day post-vaccination is 3462.6 (Table 2, Fig. 2).

Table 2

The maternal and post-vaccination antibody titre in birds immunized with Vaccine B

No.	Day 11 (vaccination day)	Day 18 (7 days post-vaccination)	Day 25 (14 days post-vaccination)	Day 32 (21 days post-vaccination)	Day 39 (28 days post-vaccination)
1	1144	271	1789	2835	3243
2	1056	318	2053	2789	4065
3	922	143	2205	2678	3812
4	1167	187	1934	2938	3784
5	1359	136	2167	2721	4122
6	1214	266	1712	3025	3591
7	1387	232	2167	3145	3233
8	935	290	2320	3323	3006
9	1141	178	1932	2890	3410
10	1098	269	2678	3144	2994
11	1277	312	2144	2935	3165
12	1321	275	2134	3096	3823
13	1026	176	2341	2678	3671
14	934	198	1854	2933	4132
15	966	265	2300	3045	3501
16	1052	143	2158	3187	3243
17	902	276	1836	3291	3127
18	1187	149	2379	2952	3238
19	1251	123	2201	3006	3009
20	905	381	2004	2862	3083
X	**1112.2**	**229.4**	**2115.4**	**2973.65**	**3462.6**
σ	**157.19**	**71.13**	**227.48**	**182.581**	**377.09**
CV%	**14.13**	**31.08**	**10.75**	**6.13**	**10.89**

Legend: X = mean; σ = standard deviation, CV% = coefficient of variation

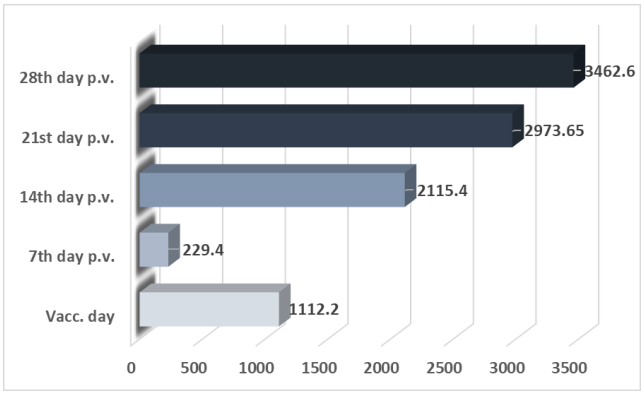


Fig. 2. Dynamics of maternal and post-vaccination antibody titres in Cobb 500 hybrids immunized with Vaccine B (average values)

Cumpănăşoiu et al. (5) and Scutaru et al. (13), had similar results in their studies. Evaluating the dynamics of post-vaccination antibody synthesis induced by attenuated vaccines, it has been observed that their level tends to increase starting from days 14-15 after vaccination, reaching maximum values in 28-30 days. The coefficients of variation obtained demonstrate the homogeneity of the vaccine effect.

The immunogenic effect of Vaccine C: The administration of Vaccine C caused a significant reduction ($P<0.001$) in the titre of maternal antibodies on the seventh-day post-vaccination (compared to the day of vaccination). From this moment, the increase in the level of post-vaccination antibodies was constant, the differences noticed between the determination made on days 14, 21 and 28 being statistically significant ($P < 0.001$) (Table 3 and Fig. 3).

Comparative evaluation of the tested vaccines

Vaccination is currently the only method to combat avian infectious bursitis that offers encouraging results (9, 17), which is why the immunogenicity and safety of anti-IBD vaccines is an aspect of major importance. Currently, a wide variety of attenuated, intermediate and "hot" strains are used in vaccines, knowing on the one hand that the attenuated ones do not offer protection against infection with highly virulent strains and, on the other hand, that slightly attenuated vaccines can induce irreversible damage to the bursa of Fabricius, affecting the immune response to other infectious diseases, even though the immune response to IBD is adequate (2, 3, 11, 15, 18).

The present study aimed to evaluate both the anti-IBD humoral immune response developed following vaccination with two live attenuated strains (composed of Vaccines A and B) and an intermediate strain (composed of Vaccine C), in a post-vaccination period of 28 days.

Comparing the results obtained after the administration of the three vaccines, it was found that they induced a similar pattern in the evolution of anti-IBD antibodies: significant reduction until the 7th day post-vaccination and constant increase until the 28th day (Fig. 4). It must be pointed out, that Vaccine C caused the most significant drop ($P<0.001$) in the level of antibodies on the seventh-day post-vaccination (average value 9.09 times lower than that recorded for maternal antibodies on the day of vaccination). This phenomenon can be explained by the fact that the viral strain in the vaccine contains the least attenuated strain.

Table 3

The maternal and post-vaccination antibody titre in birds immunized with Vaccine C

No.	Day 11 (vaccination day)	Day 18 (7 days post-vaccination)	Day 25 (14 days post-vaccination)	Day 32 (21 days post-vaccination)	Day 39 (28 days post-vaccination)
1	1213	145	1765	2732	3667
2	1009	98	1961	2876	3981
3	1143	163	1602	3245	4105
4	1234	124	2093	3127	4182
5	1465	159	1737	2934	3871
6	1324	121	2101	2720	3724
7	1067	109	1634	2971	3754
8	1291	149	1956	2765	3660
9	1007	138	1789	2668	3623
10	1432	153	2133	2976	3738
11	1166	168	1773	3359	4203
12	1298	142	1894	3478	4009
13	1382	158	1804	3121	3934
14	1456	118	1851	3420	3072
15	1278	156	1792	2643	3589
16	1197	131	2176	2697	3734
17	1371	144	1933	3208	3960
18	1322	106	2135	3154	3722
19	1350	132	2006	3007	3845
20	1236	160	1987	2679	3673
X	**1262.05**	**138.7**	**1906.1**	**2993**	**3802.3**
σ	**132.31**	**20.05**	**165.14**	**259.64**	**245.94**
CV %	**10.48**	**14.45**	**8.66**	**8.67**	**6.46**

Legend: X = mean; σ = standard deviation, CV% = coefficient of variability

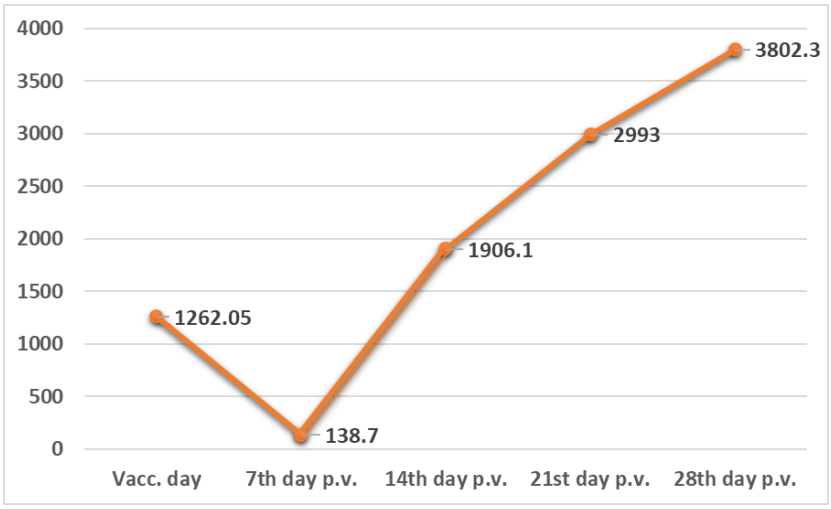
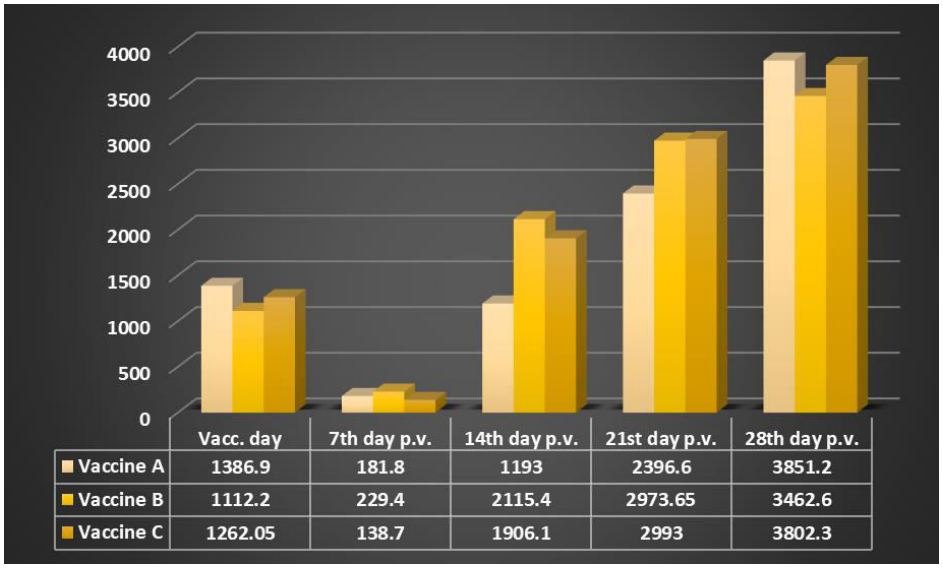


Fig. 3. Dynamics of maternal and post-vaccination antibody titres in Cobb 500 hybrids immunized with Vaccine C (average values)

It should also be stated that the level of anti-IBD antibodies obtained on the seventh-day post-vaccination does not protect against possible natural infection.

On days 14 and 21, the levels of anti-IBD antibodies, induced by the administration of Vaccine B and C, were similar (differences without statistical significance, $P>0.05$), while these results were significantly higher than those of Vaccine A ($P<0.001$), values obtained on the mentioned days (Fig. 4).

Surprisingly, this aspect is not observed 28 days post-vaccination, when the maximum titre of anti-IBD antibodies in the group tested with vaccine A (attenuated) reaches a value comparable to that recorded in the group tested with vaccine C (intermediate). At the same time, statistically significant differences ($P<0.05$) were recorded between these two titres and the maximum titre related to the group that received vaccine B, the latter being lower (Fig. 4). All these values are considered, according to the manufacturer of the ELISA kit, to be at a level that protects against infection with the infectious IBD virus.



	Vacc. day	7th day p.v.	14th day p.v.	21st day p.v.	28th day p.v.
Vaccine A	1386.9	181.8	1193	2396.6	3851.2
Vaccine B	1112.2	229.4	2115.4	2973.65	3462.6
Vaccine C	1262.05	138.7	1906.1	2993	3802.3

Fig. 4. Comparative graphic representation of maternal and post-vaccination antibody titres in Cobb 500 hybrids immunized with Vaccines A, B and C

Conclusions

Post-vaccination antibody titres induced by vaccines A, B and C showed differences, sometimes significant, during the monitored period, the most constant evolution is recorded in the case of vaccine C.

The level of antibodies against the IBD virus, recorded on the seventh-day post-vaccination (18 days of age), does not protect against natural infection.

Starting with the interval of 7-14 days and later, during the entire monitored period, which corresponds to the interval of maximum susceptibility to IBD infection, vaccines A, B and C induced the synthesis of antibody titres that ensure an adequate level of protection.

Acknowledgement

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POSTOPERATIVE OUTCOME OF DOGS WITH IVDD DISEASES IN LUMBAR AREA USING REHABILITATION METHODS

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Summary

Intervertebral disc disease (IVDD) is a common cause of neurologic dysfunction in dogs, the IVDD can lead to cervical and thoracolumbar intervertebral disc (IVD) herniation, degenerative lumbosacral stenosis, and myelomalacia. In IVDD of the lumbar spine, the animal will present unilateral lameness or paraplegia. Current treatments for IVDD in dogs include conservative treatment and surgery that requires decompression of the spine. Choosing the type of treatment was correlated with the clinical signs response of the animal to the stimulus and addition with the financial status of the owners. This study included 36 dogs, of which 24 received conservative treatment, 8 underwent surgical hemilaminectomy and 4 were euthanized. Postoperative management is recommended and may include: physiotherapy, hydrotherapy, acupuncture, and electroacupuncture. This article presents the evolution of dogs with IVDD after combining therapeutic methods with postoperative rehabilitation therapy.
Keywords: Intervertebral disc diseases, dog, treatment, rehabilitation.

Intervertebral disc disease (IVDD) is a process by which there is degeneration of the disc that lies between the two adjacent vertebrae. These discs act as shock absorbers to reduce the impact that daily activity has on the spine, and they also provide stability to the vertebral column. The intervertebral disc has a gelatinous center inside and a fibrous capsule on the outside that holds the gelatinous center in place. In animals, the disc can degenerate over time, which means that the gelatinous material will dehydrate and will turn into coarse, grainy material (3, 10). The dehydrated disc material can damage the fibrous capsule and cause it to thin. Over time, even with normal daily activities, a small hole can tear in the fibrous capsule, allowing the gritty material to push into the spinal canal where the spinal cord is located.

When this happens, the spinal cord undergoes compression leading to a degeneration of the intervertebral disc, this leads to the generation of hydraulic pressure within the disc which is radiated in all directions and which is represented by a structural defect associated with abnormal or accelerated changes seen during aging (12, 13).

Intervertebral disc degeneration is defined as a structural defect of the intervertebral disc associated with abnormal or accelerated changes observed during aging (7).

This pathology in the dog is of major importance, with a prevalence of 3.5% and a mortality of 1% (20).

Two forms of disc disease that may require surgical management have been identified: Hansen Type I (extrusion of the nucleus pulposus) and Hansen Type II (continued degeneration and protrusion of the outer fibrous layers of the annulus) (18).

Treatment methods vary widely and are represented by conservative treatment, and drug treatment in animals that have limb sensitivity and are not in a severe condition (4). Decompressive surgical techniques, in paraplegic animals that do not have limb sensitivity, cage rest, or a combination of the above (1).

The optimal treatment of a subject with neurologically complete paraplegia involves a combination of surgery decompression and supportive medical therapy (2).

When non-surgical treatment has failed, when significant neurological deficits are present and/or when pathology requires surgical treatment for symptomatic IVDD is indicated. The most common indication for surgical treatment of canine IVDD is acute disc extrusion in dogs with chondrodystrophic.

These cases are treated by surgical decompression and partial discomission by partial corpectomy, laminectomy, hemilaminectomy, facetectomy or foraminotomy of the affected disc (6, 9).

Rehabilitation methods from the use of acupuncture, electroacupuncture, and physiotherapy are represented by exercise sessions twice a day under supervision.

Patients may undergo underwater treadmill therapy with gait shaping. The speed of the treadmill is adjusted to a comfortable walking pace on the forelimbs, with the water level reaching shoulder level (8).

Age and weight were reported to have an association with the time required to regain the ability to move (17).

The aim of this study is to assess the recovery of dogs with intervertebral disc disease following the application of medical or surgical treatment and rehabilitation methods.

Materials and methods

The study was carried out in the Surgery Clinic of the Faculty of Veterinary Medicine in Timisoara in the period 2018-2022 with the inclusion of a number of 36 dogs that presented neurological deficits of the pelvic limbs. The clinical examination consisted of performing a neurological examination with testing of the following reflexes: anal, panniculus, patellar, proprioceptive, and superficial and deep pain.

Imaging diagnostics were performed, to confirm the diagnosis of spinal cord compression, by CT scans with or without contrast material. For scanning, animals were sedated using Medetomidine (20- 40 mcg/ kg), Ketamine 5 mg /kg, and 1% Propofol (3-7 mg/kg) was used for supplementation. Siemens Somatom Definition AS 64 was used to perform the scans, which allows for obtaining multiplanar sections (MPR) at a slice of 1 mm. Patients with minor clinical signs where CT scans were

performed with contrast material introduced into the subarachnoid space. As we underlined in the introduction the treatment method can be conservative or surgical.

In conservative treatment, the non-steroidal anti-inflammatory drug meloxicam (0.1 mg/kg) was administered for 10 days, combined with a gastrointestinal Famotidine (0.5 mg/kg) and a vitamin B1, B6, B12 complex (Neuromultivit) for 21 days. During this treatment period, it was recommended to limit exercise and walk the dog on a leash.

Surgical treatment consisted of hemilaminectomy and removal of the compressive disc material.

Both methods would be followed by physiotherapy, electroacupuncture, and acupuncture sessions.

At 48 hours postoperative, electroacupuncture treatment was applied every other day at Bai Hui, Shen Shu, BL23, BL24, BL40, GB34, ST36, LIV3, KID3, SI3, LIU FENG, HUA TUO JIA JI, GB29, GB 30, KID 7, SP6, SP9 electroacupuncture was applied at other acupoints traditional acupuncture was used (Fig. 1). Electrical stimulations with at 20 Hz, 80 Hz, and 120 Hz, 4,5V were conducted for 30 min by using an electrical stimulator.

Cage confinement was applied concurrently.

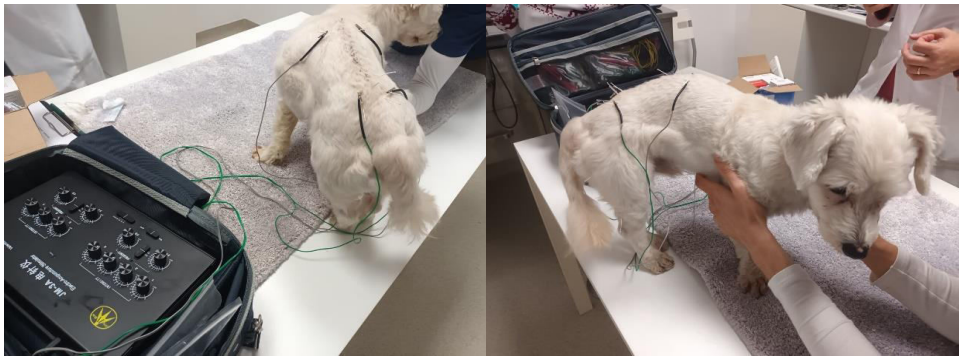

Fig. 1. Electroacupuncture (Original)

Results and discussions

Of the 36 cases examined, 4 were euthanized, 24 were treated conservatively (poor response to stimulus on limbs, costs too high for the owners) and 8 were treated surgically (partial response to stimulus).

Out of 24 patients, in the case of treatment conservative treatment, full recovery was observed in 1 week in 4 cases, after 2 weeks in 6 patients, after 1 month in 5 patients, 9 cases were left with sequelae, and 1 patient was euthanized (Fig. 2).

Out of 16 dogs treated conservatively with 8 to 10 weeks' rest and NSAIDs, only 50% of owners reported a good outcome and 25% of the dogs suffered relapse within the study period (16).

Most recently De Decker et al. reported that 17/31 dogs with IVDD (55%) were successfully medically managed with anti-inflammatory and analgesic drugs plus exercise restriction; however, 10 (32%) failed medical treatment and subsequently underwent surgical decompression, and 3 (10%) were euthanized due to progression of their clinical signs (5).

Of the 8 patients that underwent surgical treatment, after 1 week 3 have recovered, after 2 weeks 2 have recovered, after 1 month 1 recovered, and after 6 months 1 case dah recovered; there were no patients with sequelae and one patient was euthanized (Fig. 3).

Analgesics are often required for one to two weeks following surgery. Exercise should be restricted in dorsal laminectomy cases for one month and then gradually increased. Six months of restricted exercise is necessary following distraction-stabilization surgery (14).

A general opinion is that conservative treatment is of first intention, this being cage rest, limitation of activity and the use of a movements, use of harness for and the administration of anti-inflammatory drugs together with medication to counteract some of the adverse effects: hepatoprotective, gastric bandages (7).

Lumbosacral epidural injections of corticosteroids have recently been reported as a method of treatment in dogs, showing improvement in 79% of patients. For epidural corticosteroid injection treatment to be successful, patients must not have proprioceptive deficits in the hindlimbs or urinary and fecal incontinence (15, 19).

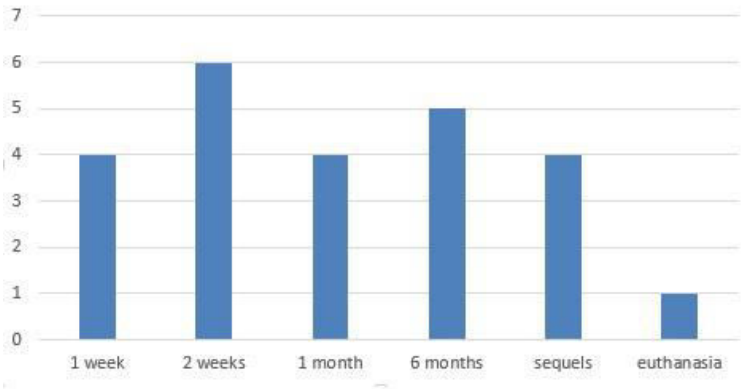

Fig. 2. Evolution of patients after conservative treatment

Comparing recovery rates with previous studies is difficult because of the many variables, such as study Inclusion criteria, the duration of absence of deep pain perception and loss of motor function, the type of surgery performed, and the definition of a successful outcome (11).

Animals should improve within 2 to 3 weeks and significant improvement should be noticed in a month.

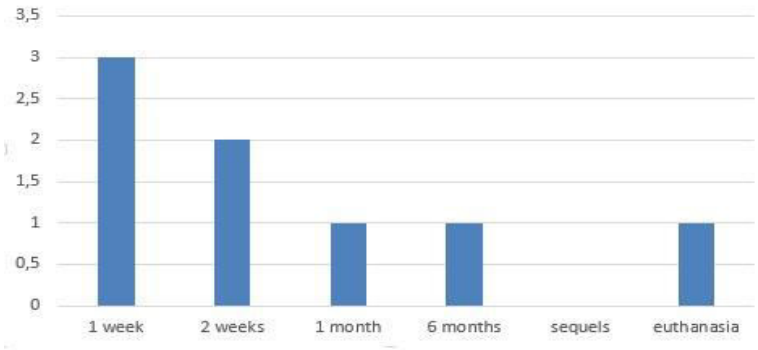

Fig. 3. Evolution of patients after conservative treatment

Conclusions

Patients who did not respond to painful stimuli didn't fare well in terms of recovery.

Patients who reacted to painful stimuli during the initial examination recovered completely within a short period.

Medical treatment has a good outcome in acute hernias and those where a small amount of disc was extruded.

The combination of this type of treatment with physiotherapy, acupuncture, and electroacupuncture has improved patient recovery in chronic cases.

Hemilaminectomy is an effective method of treating acute cases in which the disc material has not mineralized and/or adhered to the vertebral body.

Physiotherapy, acupuncture, and electrosurgery have reduced the recovery time.

Physiotherapy increases muscle tone and helps the animal to be able to support its weight.

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SEROPREVALENCE OF *TOXOPLASMA GONDII* INFECTION IN WILD BOAR IN TIMIS COUNTY - PRELIMINARY RESULTS

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Summary

Toxoplasmosis is a protozoan disease caused by *Toxoplasma gondii*, a worldwide distributed, intracellular apicomplexan protozoan, that affects all homeothermic animals, including humans. The present study aimed to establish the seroprevalence of *T. gondii* infection in wild boars in Timiș County. The samples were collected from 85 wild boars which were either hunted or found dead on the hunting grounds. The samples were collected during the period 2020-2021, from 29 hunting funds from Timiș County. Serum samples were examined through the ELISA test, using the ID-VET Screen Multi-species kit for IgG anti-Toxoplasma antibodies. Out of 85 examined samples, 80% showed anti-Toxoplasma IgG antibodies. All 68 positive samples were taken from adult wild boars, none from young or subadult animals. There were no significant differences between seropositivity in males compared to females. Significant differences were observed between the different age groups. Considering all the animals from the 29 hunting funds, we found positive evidence in animals from 26 hunting funds (89.7%) while the animals from the other three hunting funds (10.3%) were negative.

Keywords: wild boar; *Toxoplasma gondii*, ELISA, seroprevalence.

Toxoplasmosis is one of the most common parasitosis in humans and animals, ranking in the top three worldwide (3). *Toxoplasma gondii* is considered one of the most parasitic parasites known to date. It has a heteroxenic life cycle and can infect all warm-blooded animal species (mammals and birds), including humans (3, 9 - 17). The parasite can be found in any part of the world, which demonstrates its medical and veterinary importance as it can cause abortion and congenital disorders in intermediate hosts (1, 26, 33, 44). Due to its zoonotic importance, *T. gondii* is the most studied of the coccidia.

In the transmission of toxoplasmosis, carnivorism has a very important role. Through carnivorism, one of the links of the biological cycle is avoided: the cat. It seems that the sheep and the pig play the most important role in this mode of transmission. In pigs, infection occurs by eating uncooked food scraps or rodents (7, 18, 19).

The goal of our study was motivated by reports of a rise in toxoplasmosis cases in humans and animals worldwide. In light of this, the purpose of the current study was to determine the frequency of *T. gondii* infection in wild boars in western Romania.

Materials and methods

The animals studied were represented by 85 wild boars found dead or shot in the period 2020-2021 on hunting grounds in Timis County. The biological material on which the work was carried out was represented by 85 blood samples collected from the wild boars and sent by the representatives of AJVPS Timiș to DSVSA for further analysis. The present study aimed to establish the seroprevalence of *T. gondii* infection in wild boars in Timiș County.

The collected blood was left to decant for serum expression and kept in the freezer until the samples were processed in the Parasitology and Parasitic Diseases Laboratory of the Faculty of Veterinary Medicine in Timisoara.

Serum samples were examined by indirect ELISA using ID-VET Screen Multi-species kits for the detection of specific anti-toxoplasma Ig G antibodies resulting from *Toxoplasma gondii* infection. The kit can be used for the determination of anti-toxoplasma antibodies in the sera of ruminants, pigs, and cats.

The plate was analyzed in a microplate reader at 450 nm wavelength. The optical densities obtained from the plate reading were interpreted according to the manufacturer's formula. Values above 200% were considered strongly positive, values between 50 and 200% were rated positive, those between 40 and 50% had an uncertain status, and values below 40% were considered negative.

Data analysis was performed by GraphPad, QuickCalcs, Fisher's exact test, and Office Excel 2016.

Results and discussions

Of the 85 serum samples processed from wild boar, 68 (80%) showed IgG anti-Toxoplasma antibodies. Antibody titer values ranged from 31.05 to 133.18 and for positive samples values ranged from 68.25 to 133.18.

After analyzing the data obtained, it was found that of the 17 negative wild boars 13 were juveniles, four were of unknown age, 11 were males and 6 were females. Concerning the hunting grounds of origin, the negative animals were from 10 Bărăteaz, 16 Șarlota, 17 Nadăș, 37 Visag, 5 Igris, and 75 Becicherecu Mic.

The 68 samples positive for *Toxoplasma* antibodies were recorded in adult boars, none in juveniles or subadults. The prevalence on hunting grounds ranged from 50 to 100%.

All the hunting grounds from which the wild boar originated, except 10 Bărăteaz (50%), 37 Visag (50%), and 17 Nădaș (57.1%) had 100% prevalence. Overall, of the 29 game funds, six had negative samples, 26 had positive samples (89.7%) and three had both positive and negative samples. Consequently, three (10.3%) of the 29 hunting grounds were negative. Indeed, the number of wild boars hunted was also small on these hunting grounds, namely six (16 Șarlota), one (5 Igis), and two (75 Becicherecu Mic).

Regarding seropositivity by sex, it was found that out of the 68 samples from wild boars in Timis, 55 were from males (81%), eight were from females (12%) and 5 were not identified (7%).

The same analysis carried out on the negative samples reported on the sex of the animals revealed that out of the 17 samples from wild boars in Timis 11 were from males (65%) and six from females (35%).

Statistical analysis did not reveal significant differences between seropositivity in males and females, but strongly significant differences were recorded in the age groups, youth, and adults, with youth being the negative age group. This aspect is very important in the epidemiology of infection in wild boar and can be explained by the fact that infestation occurs over time through the consumption of infesting elements, oocysts, or tachyzoites and bradyzoites.

For Timis county, but also the neighboring counties, the information obtained is very important regarding toxoplasma infection in this area. The increased zoonotic risk of this protozoan transmissible through the consumption of insufficiently cooked meat leads us to be concerned about the actual infection situation in humans.

Moreover, the very high prevalence of 80% in wild boar is a warning signal of the extent of environmental infestation with oocysts and the massive infestation of wild animals. This should scare us even more given the fairly frequent consumption of game meat, particularly wild boar meat.

This study shows the absolute need to follow good husbandry and feeding practices to reduce the risk of transmitting *T. gondii* infection to humans and other animals.

Studies on the same geographical area have also been carried out by Hotea et al., 2010, (25) when they identified a prevalence of 94.23%, but also by Grema et al., 2015, 2016, who obtained a seroprevalence of *Toxoplasma gondii* infection in wild boars from Timiș and Caraș Severin of 61.33% and 33.33%, respectively (23, 24).

Most studies worldwide are carried out in domestic pigs, and the results are among the most diverse, ranging from 2.6% - 33% in the Netherlands, in Italy 16.3%, In Germany, 5.6%, In Serbia, out of 605 serum samples examined, 28.9% (22, 28, 29, 42, 43, 45).

By indirect immunofluorescence, a prevalence of 26.2% in wild boar was established in the Czech Republic and 8.1% in the Slovak Republic (2, 5). In Spain, the prevalence of *Toxoplasma gondii* infection in wild boar was 38.4% and in Japan, it ranged from 5.6% in 1999 to 0% in 2006 (20, 21, 34-36).

In the USA, the prevalence of toxoplasma infection ranged from 27.7 to 88.6%, and in Brazil from 8.54 to 66.67% (2).

Omnivores (pigs) often become infected by eating small rodents (dead or diseased mice), which carry toxoplasmic cysts. On some farms, it has been possible to link the percentage of infected murids to the percentage of toxoplasma seropositive pigs. In addition, pigs under certain circumstances become cannibalistic by biting their tails or ears (codophagia and otophagia) (42, 43).

Tissue cysts of *T. gondii* in game meat (wild boar) are also considered a source of infection for humans (6, 27, 31, 37, 40, 41). Hunters and their families can also become infected during evisceration and butchering of the game (27, 35, 36, 40).

In Europe and the US, pigs have been considered a major source of infection for humans (7).

Anti-T. gondii antibodies were detected by ELISA in 260 out of 656 wild boars (40%) with a prevalence of 26% in piglets (72/279) and 50% prevalence in juveniles and adults (188/377) in several districts of the Czech Republic. The total seroprevalences of the region ranged from 32% - 59%, with a significantly higher prevalence in the Havlíčkův Brod region (59%). Statistically significant differences (p-value <0.05) resulted between 2 age categories and between 9 regions, with significant variability in the Havlíčkův Brod region. Seroprevalence correlated positively with farm density, but without any statistical significance (38).

Food safety regulations require the control of protozoa in meat intended for human consumption. Meat from wild boar (*Sus scrofa*) can be a source of potentially zoonotic pathogens. A seroprevalence of 23.8% (688/2881) of anti-Toxoplasma *gondii* antibodies and 72.2% (662/910) of *Sarcocystis* prevalence were detected in wild boar hunted in the south-western areas of Spain (8).

In an 11-month survey, a total of 882 serum samples were obtained from boars raised in three cities (Jilin, Siping, and Baishan) in Jilin Province, northeast China and tested for *T. gondii-specific* antibodies. Using the modified agglutination test and a cut-off titer of 1:25, the prevalence of *T. gondii* infection in the samples examined was 10.0% (88 of 882). The highest seroprevalence was observed in animals from Jilin City (15.3%, 43/281), followed by Siping (11.4%, 30/263) and Baishan (4.4%, 15/338). Logistic regression analysis revealed a significant correlation between the geographical region investigated and *T. gondii* infection. In addition, the prevalence was higher in females compared to males and the highest prevalence was detected in piglets (4).

A total of 1279 blood samples from 41 regions were collected from apparently healthy wild boar during the three hunting seasons (September-February) 2014-2015, 2015-2016, and 2017-2018. Of these sera, 461 samples tested positive with a commercial indirect ELISA kit for *T. gondii*, and the total apparent and adjusted seroprevalence was estimated to be 36.0% (95% confidence interval [CI], 33.4-38.7) and 31.3% (95) % CI, 33.1-38.9), respectively. Seroprevalence was significantly higher in juveniles and adults than in piglets ($P < 0.05$); however, no significant difference by sex was observed (30).

Serum samples from 101 wild boars collected in 2016-2018 from five different locations in mainland Denmark, Jutland, were examined for anti-T. *gondii* antibodies. Samples were analyzed using a commercial ELISA test. 28 samples (27.7%) of the 101 boars tested positive. The odds of a boar testing seropositive were higher if it was sampled during the 2017-2018 hunting season than during

2016-2017 and if it was reported to be at least 1 year old than if it was younger (two-variable logistic regression model: odds ratios 17.5 and 3.9, respectively (32).

Conclusions

The prevalence of *Toxoplasma gondii* infection in wild boar was 80%, an alarming figure, especially for hunters' families and consumers of undercooked or raw products.

The geographical spread of the hunting grounds is wide as 89.66% of them showed positive samples.

The age of boars is a risk factor for toxoplasma infection whereas sex did not influence seropositivity.

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PREVALENCE OF *TOXOCARA* INFECTIONS IN DOGS FROM RURAL AREAS IN SOUTHWESTERN OLTENIA AND ASSOCIATED RISK FACTORS

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Summary

The roundworm *Toxocara canis* is the most common zoonotic parasite of the dog (natural reservoir) in many areas of the world, including Romania, and is important for public health. The present study aimed to identify the prevalence and associated risk factors for *Toxocara* infections in dogs from rural areas in Southwestern Oltenia. A total of 100 faecal samples were collected from dogs, in three rural areas, from Gorj and Vâlcea counties, aged between 3 months and 14 years. To detect helminth eggs, all samples were examined by direct smear and faecal flotation method (Willis). All owners have completed an epidemiological file with questions related to the dog's lifestyle, environmental hygiene, contact with animals or humans, and the type and mode of feeding and watering. There were questions about the number of deworming, the products used, and the physiological status. Data analysis was performed by GraphPad, QuickCalcs, Fisher`s exact test, and Office Excel 2016. The overall prevalence of *Toxocara* infections was 73% (73 out of 100). Out of the total number of dogs, 83% were dewormed with 8 commercial products, 62% consume commercial food, 65% directly from the ground and come into contact with other animals, 100% come into contact with humans, 52% are free in the yard, and cleanliness in the living environment is not done at 25%.

Keywords: dogs, *Toxocara* spp., flotation method, prevalence, risk factors.

Discovered in 1782 (12), the roundworm *Toxocara canis* is the most common parasite of the dog (natural reservoir) in many areas of the world, including Romania, and due to its zoonotic potential is a common concern for both veterinary medicine and medicine human.

Infestation in humans is achieved by direct contact with animals that remove larval eggs with faeces, but also by eating insufficiently cooked meat or even through unclean hands contaminated with eggs (12). Once penetrated the human body, this parasite will be located in various organs and tissues, generating the so-called *larva migrans visceralis* syndrome (3, 4). Toxocarosis may have an asymptomatic course or may be accompanied by characteristic clinical signs (general, cutaneous, respiratory, digestive, and cardiac signs) (9).

In the case of optimal microclimate conditions, *Toxocara canis* eggs last in the external environment for several months to several years (9).

This increased prevalence identified among canids may be based on the low percentage of routine diagnoses of veterinarians and the lack of sanitary-veterinary education among the population.

The present study aimed to determine the prevalence of the parasite *Toxocara canis* in Gorj and Vâlcea counties in the southwestern of Oltenia and respectively the associated risk factors for *Toxocara* infections in dogs.

Materials and methods

Two coprological examination methods: direct smear and faecal flotation method (Willis) of 100 samples of faeces collected in plastic bags from dogs aged between 3 months and 14 years were performed. The dogs subjected to the study came from different localities of Gorj and Vâlcea counties.

An epidemiological file was completed for each dog, which included the following details: breed, age, sex, dog's lifestyle, environmental hygiene, contact with animals or humans, and the type and method of feeding and watering. Data analysis was performed by GraphPad, QuickCalcs, Fisher's exact test, and Office Excel 2016.

Results and discussions

The 100 rural dogs studied were divided into 4 age categories as follows: 0 months - 1 year (31 dogs), 1 - 4 years (34 dogs), 4 - 9 years (29 dogs), and 9 - 14 years (6 dogs).

Following the parasitological examination of the faecal material samples, the following types of classes were highlighted: Nematoda: *Toxocara canis, Uncinaria / Ancilostoma, Trichuris vulpis,* and Cestoda: *Dypilidium caninum*, respectively.

The prevalence of the parasitic elements identified was as follows: *Toxocara canis* eggs: 67% (67/100) (monoparasitism); *Toxocara canis* and *Ancilostoma / Uncinaria* eggs: 3% (3/100); *Toxocara canis* and *Trichuris vulpis* eggs: 3% (3/100); *Trichuris vulpis* eggs: 1% (1/100); *Dypilidium caninum* eggs: 1% (1/100).

Of the total prevalence of 73%: for the age range: of 0 months - 1 year out of a total of 31 dogs, only 27 dogs are positively confirmed, the prevalence is 87% (27/31).

In the age category 1 - 4 years, only 21 canids were diagnosed positively, with a prevalence of 61% (21/34).

Out of the 29 dogs aged between 4-9 years, only 20 cases were positive, with a prevalence of 68% (20/29).

In dogs aged between 9 and 14 years out of a total of 6 dogs, only 4 were positive for *Toxocara canis*, the prevalence being 66% (4/6).

Therefore, the highest positivity was registered in the age category 0 -1 year. The transplacental transmission was also confirmed in the current study in a female with 4 puppies, of which only two were confirmed positive for *Toxocara canis*.

Out of the 100 dogs examined, a pregnant female was also diagnosed positively. In this case, because the possibility of infestation of puppies is quite

high, it is possible to use anthelmintics until the age of 21 days for puppies to destroy parasites that have not reached maturity. The treatment will be repeated at 60 days and 90 days, respectively at 6, 9, and 12 months (3).

Of the total number of dogs, 83% were dewormed with 8 commercial products, 62% consume commercial food, 65% directly from the ground and come into contact with other animals, 100 % come in contact with humans, 52% are free in the yard, and the cleanliness in the living environment is not done at 25%.

Out of a total of 83 dewormed dogs, only 23 were dewormed both internally and externally. Of the 23 dogs, only 9 were positively confirmed *Toxocara canis*, the prevalence being 39% (9/23).

Of the 8 commercial products used for deworming animals, 4 were used for internal deworming. Out of a total of 43 dogs dewormed with these drugs, a number of 23 were positive, with a prevalence of 53, 48% (23/43).

The receptivity of animals does not seem to be influenced by age: some authors identify a high prevalence in dogs under 1 year of age, and others in those over 1 year of age (7).

The prevalence of parasitism with *Toxocara canis* in western Europe is between 3.5 and 34% (12).

The prevalence of nematode *Toxocara* spp. in dogs in Iran was 24,2% (5).

In Timisoara, the prevalence value for *Toxocara canis* was 17,30% compared to northern Greece, where the prevalence for the same species was lower: *Toxocara canis* was 12,8% (7, 15).

At the level of Timiș County in the period 2009 - 2011, there was a prevalence of 4,37% for *Toxocara* spp. (18).

Luca et al., 2019, highlight the prevalence rate of nematodes with zoonotic risk in the study conducted on a total number of 207 dogs (both owned and unowned) from Timișoara. for owned dogs the prevalence of gastrointestinal nematodes was *Trichocephalus vulpis* 42.85%; *Toxocara canis* 36.73%; *Ancylostoma caninum* 14.28%; *Toxascaris leonina* 4.08%. For stray dogs, the recorded prevalence was for *Ancylostoma caninum* 97%; *Toxascaris leonina* 32%; *Toxocara canis* 19%. The dogs were classified into four age categories, and within them, the highest parasitism rate was in the category: 0-6 months. From the point of view of breeds, parasitism proved to be high in large and mixed breeds (7).

During the period 2017-2020, Luca et al., 2021, studied the population of carnivores (dogs and cats) in different areas of Romania. In Timișoara, Timiș county, samples were taken from 219 animals, of which 176 were dog faeces samples. Among the parasite species identified, *T. canis* had a prevalence of 20.45%; *Ancylostoma*/ *Uncinaria* 17.04%, *Toxascaris leonina* 1.7%. Relative to age, the categories 0-6 months and 1-3 years had the most positive cases. Male dogs had the most positive results, 43.56%. In the case of those of large size, the recorded prevalence was 41.66%, medium size 35.61%, and small 26.19%. The prevalences for Hunedoara County were 53.62%, followed by Gorj at 38.88% and Timiș at 35.89% (6).

A study carried out in 2012-2013 by Mederle et al. (10), highlighted the presence of 97 neutered dogs (out of a total of 171) of one parasitic element with zoonotic potential. Three methods were used to examine the samples: Willis flotation, McMaster quantitative, and direct Lugol coloring. The recorded

prevalence was as follows: for *T. canis* eggs 85.56%, for *Toxascaris leonina* eggs 3.09%, for *Ancylostoma* / *Uncinaria* eggs 62.88% (10).

Ursache et al. in a study conducted in Cluj County, Romania, over two years, a total of 155 faecal samples were examined by the Willis flotation method obtained a total prevalence of 67.1%. Of this total, 34.8% was occupied by the parasite *Toxocara canis*. Monoparasitism was found at a rate of 20%, and in the case of polyparasitism, it recorded a value of 14.8%. These values of the prevalence of the zoonotic nematode *T. canis* were influenced by two factors: the age of the animals (the most affected: puppies) and respectively by the lack of deworming. In recently dewormed dogs, the prevalence was lower (12.3%) compared to non-dewormed animals (51.1%). The prevalences, in the case of the other species of gastrointestinal parasites, were: *T. vulpis*: 20%; *Ancilostoma* / *Uncinaria*: 10.4% (20). Furthermore, in the same geographical area, Mircean et al., 2017, reported that *T. canis* infection is prevalent in dogs, particularly pups, with a significant impact on the well-being of affected animals and, not least, public health (11).

Between 2006 and 2007, Tudor conducted a study to assess the prevalence of toxocariasis in dogs and, respectively, the risk of infection of children with the *T. canis* parasite. Faecal samples, 173 in number, were taken from three public parks in Bucharest, Romania. A prevalence of 42.77% was recorded in the case of positive samples for parasite eggs or oocysts, and for the positive samples of *T. canis*, the prevalence value was 17.92% (19).

In another study carried out in southern Romania, a *Toxocara canis* infestation prevalence of 4.26% was identified (17).

A study conducted in Mampong, Ashanti, Ghana found a prevalence of monoparasitism of 53 % of which *Toxocara canis* was 18.8%, and the associated favoring factors for *Toxocara canis* infection in dogs were age and region of origin animals (2).

In dogs from Chisinau, there was a prevalence of 53.6% for the parasite *Toxocara canis*, this value being related to youth (12).

A source of infestation for humans is the fur of animals on which *Toxocara canis* eggs can be found. Following a study conducted in Egypt, a percentage of larval eggs between 10.7 and 26% were identified on the fur of animals. Also in the same study, the nematode *Toxocara canis* was identified in the faecal samples, with a prevalence between 21.3 and 35.8% (4).

Between 2008 and 2010, a study conducted in Bucharest highlighted the following prevalences for *Toxocara* spp: 17% for *Toxocara canis* and 4% for *Toxascaris leonina*, the most affected age group being dogs under 1 year old (1).

According to a study carried out in the Netherlands, the following was concluded: between 4.4 and 76.2 % of the faecal samples analyzed were positive for parasitism with *Toxocara* spp. Also in the same study, it was confirmed that the animals were positive for the presence of *Toxocara* spp. eggs on the fur were also positive for the faecal samples examined (13).

Globally, the prevalence of *Toxocara canis* was 10.8% for Europe, North America: 11,1%, South America: 10,9%, Africa: 18,5%, Eastern Mediterranean: 19,2%, Southeast Asia: 11,9%. Concerning the age of the animals, the highest prevalence value was identified in dogs under 12 months of age, and in terms of

sex, males were in a high number. Also, in the same study it was concluded that, worldwide, over 100 million dogs are positive for *Toxocara* spp. (16).

As dogs are the most important host for *Toxocara canis* - a nematode with a widespread zoonotic potential and one of the main causes of human toxocarosis (16) a protocol accessible to everyone, from animal owners to veterinarians or humans, should be considered how we can reduce the number of confirmed positive animals, which would also lead to a decrease in the prevalence of toxocarosis in humans. A general but very important measure is the health education of animal owners (correct and timely de-helminthization - based on a coproscopic examination performed by a veterinarian, hygiene in dog shelters, daily cleaning of bedding, regular hygiene of the cage, and pen, disinfection of surfaces contaminated using solutions with ovicidal action) (8).

Complete eradication of the roundworm *Toxocara canis* is impossible since it is biological cycle is complex and there are multiple ways of infestation: transplacental, larval eggs (especially in youth), paratenic hosts, or milk, the latter two being less important (3).

Conclusions

The prevalence of *Toxocara canis* roundworm in dogs from Gorj and Vâlcea counties was 73%, with an increased value within the age category 0 months - 1 year (87%) and a minimum value for dogs aged between 1 - 4 years (61%). In the context of the results obtained and, in the situation, when dogs are considered the sentinel host for the occurrence of *T. canis* infections in humans, the assessment of the high prevalence in the canine population under study, makes it imperative to recommend effective parasitological control of the disease.

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CHARACTERISATION OF THE ANTIMICROBIAL BEHAVIOUR OF SOME BACTERIAL STRAINS ISOLATED FROM DOGS WITH CHRONIC OTITIS

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Summary

Canine chronic otitis is a pathological recurrent or persistent condition characterised by inflammation of one or both ears resulting from long-term action of the primary, predisposing or perpetuating factors. Numerous bacterial species act as perpetuating factors, some of them such as *E. coli*, *S. aureus* or *P. aeruginosa*, being able to form a biofilm, so the therapeutic results depend on the possibility of destroying this biofilm. Moreover, some isolated strains involved in the aetiology of chronic otitis in dogs seem to be multidrug-resistant bacteria. The study was carried out on 16 dogs with chronic otitis, the bacterial aetiology being highlighted only in 12 cases from those taken into the study. The bacterial isolates were classified into species and genera by the API system (API 20NE, API 20E and API Staph), so it was identified *E. coli* (three isolates), *P. vulgaris* (two isolates), *S. aureus* (two isolates) and *P. aeruginosa* (five isolates) strains. The Kirby Bauer disc-diffusion method was used to test the antimicrobial efficacy of the isolates. All isolates showed resistance to 6 antimicrobials: amoxicillin/ clavulanic acid, kanamycin, ampicillin, ceftiofur, gentamicin and oxacillin. The only effective antimicrobial substance against all P. aeruginosa strains was imipenem. All *E. coli* and *P. vulgaris* isolates were susceptible to ciprofloxacin and flumequine, antibiotics with evident efficacy against both *S. aureus* isolates. However, the strains of *S. aureus* were also sensitive to imipenem and cefalexin.

Keywords: canine chronic otitis, ears infection.

Otitis externa is a multifactorial inflammatory disease of the external ear canal, including the ear pinna, that can evolute acute or chronic. Chronic otitis externa is a persistent or recurrent otitis lasting for three months or longer that produces changes, including glandular hyperplasia, glandular dilation, epithelial hyperplasia, and hyperkeratosis (1, 5). These changes usually result in increased cerumen production, which contributes to an increase in the local humidity and pH of the external ear canal, the predisposing factor for secondary infection (1, 5, 16). The frequency of pathogens' involvement in dogs' chronic otitis varies from one study to another. However, various bacterial species seem to be implicated in the aetiology of this pathology, such as *Staphylococcus spp.*, *Pseudomonas*, *Proteus*, *Enterococcus*, *Streptococcus*, and *Corynebacterium* (10, 15, 17). Besides the fact that these species are currently characterised by multiple antibiotic resistance that appeared as a result of the abundant and irrational use of antimicrobial substances,

there are also species capable of determining biofilm formation. So, *Staphylococcus* and *Pseudomonas* are the species that can produce biofilm, respectively, with the persistence of infection despite adequate therapy (18). To be efficacy, the treatment needs to disrupt the biofilm, and antimicrobial therapy must combat the infection (1, 6, 18).

All this being exposed, it is understandable why an appropriate treatment must be instituted immediately after establishing the diagnosis, in the acute phase of the disease, to avoid chronicity.

Materials and methods

For the study, it was examined 16 dogs with chronic otitis. All cases were clinically examined by methods of observation, palpation and thermometry, and after the general clinical examination of the patient, samples were collected to carry out the bacteriological examination for identification of the bacterial aetiology of the disease. The symptoms varied from one case to another (Fig. 1), from the simple auricular congestion hyperplasia or stenosis of the auricular conduct with or without sero-purulent secretion.

Fig. 1. Clinical signs of chronic otitis

Special attention was paid to the anamnesis because the history and the application of previous treatments could alter the result, with the appearance of a false negative result if the patient is undergoing treatment. Moreover, the local administration of some antimicrobials could cause the appearance of bacterial strains resistant to the antibiotic used.

For the bacteriological examination, primary inoculations were performed on BHI (Brain Heart Infusion) broth; then passages were performed on nutrient agar, Levin agar, and 10% blood agar. All samples were incubated for 24 hours, at a temperature of 37°Celsius, under aerobic conditions.

Based on the biochemical characteristics of the isolated strains, it was possible to classify the bacteria into species and genera. The biochemical characterisation was performed using API systems as follows: API 20E and API 20NE for Gram-negative bacteria and Api Staph Gram-positive bacteria.

To characterise the antimicrobial behaviour of bacterial strains isolated from otic secretions, the qualitative disc-diffusimetric method, Kirby-Bauer, was used. This method is based on the property of antimicrobial substances to diffuse in a solid culture medium (Mueller-Hinton), on which the bacterial culture to be tested is passed. Thus, based on the diameter of the inhibition zone produced by the antibiotic, related to the interpretive table provided by CLSI/2009 (Clinical Laboratory Standard Institute USA/2009) (19), the strains are classified into various interpretation categories, as follows: sensitive (S), intermediate (I) and resistant (R). The following antimicrobials were chosen to be tested: gentamicin (GMN- 10 µg), penicillin (PEN- 6 µg, respectively 10 IU), oxacillin (OX- 1 µg), kanamycin (KAN- 30 µg), ampicillin (AMP- 10 µg), cefaclor (CEC- 30 µg), amoxicillin/ clavulanic acid (AMC 20/10 µg), oxytetracycline (OT- 30 µg), flumequine (UB - 30 µg), ciprofloxacin (CIP 5 µg), ceftiofur (EFT - 30 µg), imipenem (IPM 10 µg), cephalexin (CXN - 30 µg), streptomycin (S - 10 µg).

Results and discussions

Of the 16 dogs with chronic otitis, bacterial aetiology was highlighted in 12 cases. Based on the biochemical characteristics of the isolates, it was found that the strains isolated were *E. coli* (three of the 11 isolates), *P. vulgaris* (two of the 11 isolates), *S. aureus* (two of the 11 isolates) and *P. aeruginosa* (five of the 11 isolates) strains.

The antimicrobial behaviour of the isolated strains is presented in Fig. 2.

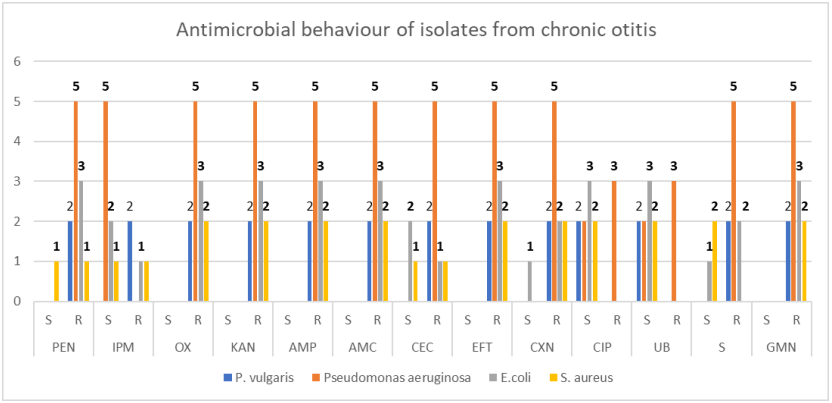

Fig. 2. Antimicrobial susceptibility and resistance of isolated strains from dog otitis

As is observed in Fig. 2, all the isolated strains from chronic infections of ears in dogs showed resistance to six antimicrobials: amoxicillin/ clavulanic acid, kanamycin, ampicillin, ceftiofur, gentamicin and oxacillin. In addition, all *E. coli* and *P. vulgaris* strains showed resistance to penicillin, and all strains of *P. aeruginosa* were resistant to penicillin, cefaclor, cephalexin and streptomycin. Besides the antibiotic mentioned above, for which all the strains were resistant, both strains of *S. aureus* presented resistance to cephalexin.

Regarding the sensitivity of the strains, it was observed that all the strains of *P. aeruginosa* were sensitive to imipenem, while two of them were sensitive to ciprofloxacin and flumequine. All *E. coli* and *P. vulgaris* strains were susceptible to ciprofloxacin and flumequine. Both *S. aureus* strains were susceptible to cephalexin, ciprofloxacin, flumequine and streptomycin.

Studies from the literature demonstrated different antimicrobial behaviour, dependent on the isolated strains (2, 3, 4, 7, 11, 12). In their study, Lyskova et al., (13) demonstrated that all bacterial isolates from otic secretion were most susceptible to gentamicin (13). Kown et al. (12) demonstrated that the percentage of resistant *P. mirabilis* strain was only gentamicin 22% (12). Eliasi et al. (8) found a low percentage of resistant strains isolated from canine otitis to gentamicin (18%) (8). These reports differ from our results, demonstrating that all isolated strains were resistant to gentamicin. However, Girlich et al. (9) showed that the percentage of *Proteus spp*. and *P. aeruginosa* colonies resistant to gentamicin was similar among the four genera of bacteria. More, the study demonstrated higher percentages of the same colonies resistant to neomycin, azithromycin and cephalexin (9). The resistance to cephalexin was demonstrated in our study, too.

The present study showed a high percentage of strains sensitive to fluoroquinolones. The results can be explained by the fact that canine otitis treatment consists of antimicrobials, such as penicillins, cephalosporins, aminoglycosides, macrolides, phenicols, polymyxin as the first choice and only as a second choice the administration of fluoroquinolones (9, 14, 20). In contrast, Kown et al. (12) found a high percentage of *P. mirabilis* strains resistant to ciprofloxacin, respectively 53% (12). Another survey about the antimicrobial susceptibility of Pseudomonas spp demonstrated over 43% resistance to enrofloxacin (6). Regarding the *Staphylococcus spp*. resistance, studies showed a low percentage of sensitive to β-lactams and cephalosporins (6), similar to the present study.

As our results, other studies demonstrated a low proportion of isolates resistant to imipenem (6%) (8).

The resistance of bacterial strains isolated from dogs can be transmitted to humans, especially due to close contact between them. Moreover, most of the strains isolated from carnivores are ubiquitous bacteria, which create pathological processes in immunosuppressed organisms or act as association flora (1, 5, 7). In most countries, otitis is treated empirically following the establishment of the diagnosis in correlation with the clinical signs, usually by administering antibiotics and corticosteroids (5). The necessity of bacteriological examinations is highlighted

in studies demonstrating the emergence of multidrug-resistant bacteria.

Conclusions

All isolates from chronic otitis in dogs were characterised by increased antibiotic resistance to common antimicrobial substances used in medical practice. Thus, all of them were resistant to amoxicillin/ clavulanic acid, kanamycin, ampicillin, ceftiofur, gentamicin and oxacillin. The only effective antimicrobial substance against *P. aeruginosa* strains was imipenem. The *E. coli* and *P. vulgaris* isolates were susceptible to ciprofloxacin and flumequine. The strains of *S. aureus* were sensitive to imipenem, flumequine and cefalexin.

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MANUKA HONEY - AN ALTERNATIVE TO ANTIBIOTICS FOR THE TREATMENT OF INFECTIONS CAUSED BY PYOGENIC BACTERIA

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Summary

Pyogenic infections are one of the most common clinical entities observed in humans and animals, caused by bacteria such as Staphylococcus aureus, Streptococcus pyogenes, Proteus spp., E. coli, Klebsiella spp., Pseudomonas spp., Acinetobacter spp., C. perfringes and Bacteroides. Prompt and aggressive antimicrobial therapy is required to reduce the complications associated with these infections. However, some of the pyogenic bacteria, especially S. aureus and P. aeruginosa strains, are included in the global priority list of antibiotic-resistant bacteria from WHO/OMS, and the reason for finding alternative treatments, especially in localized infections, is required. The present study aimed to highlight the antimicrobial activity of manuka honey against S. aureus (11 isolates) and P. aeruginosa (18 isolates) strains. The strains were isolated from dogs' otic secretions and purulent skin lesions and confirmed using the API 20NE and API Staph systems. The antimicrobial activity of manuka honey, MGO 500, was determined by the microdilution method at concentrations of 10%, 15%, 20% and 25% and the results were expressed in OD (optical density). The obtained results demonstrated that the lowest concentration of manuka honey taken into the study presented antimicrobial efficacy against all isolates of S. aureus, but not against P. aeruginosa. The MIC (minimum inhibitory concentration) value of manuka honey against all 18 P. aeruginosa strains was 20%. Even if the MIC value is different, lower for S. aureus than for P. aeruginosa, manuka honey can represent an alternative treatment method in infections produced by these two bacterial species.

Keywords: manuka honey, pyogenic bacteria, treatment.

Pyogenic bacteria represent the usual cause of suppuration, being one of the most common clinical entities observed in humans and animals, involving numerous bacterial species such as S. aureus, S. pyogenes, P. aeruginosa, Proteus spp., E. coli, Klebsiella spp. and other. All these pathogens can cause variable pathologies, from simple abscesses to cellulitis, suppurative lymphadenitis and even pyomyositis (8, 18). Prompt treatment is essential to avoid severe complications, such as sepsis. Although the choice of antimicrobial substances with increased effectiveness is the principal objective in treating suppurative infections, it is still quite challenging to approach because it must be correlated with the evolution and severity of the pathology. This affirmation is sustained by the fact that OMS/WHO included some of the pyogenic bacteria, such as S. aureus and P. aeruginosa, in the global priority list of antibiotic-resistant bacteria (15). In this context, finding

alternative methods of treating suppurative skin infections, at least in the case of mild forms, is desirable.

The antimicrobial activity of honey has been intensively researched over time. However, even though the antimicrobial activity against various bacterial isolates strains had been demonstrated, the mechanism of the actions has not been completely elucidated (3, 14, 17). Sugar content, endogenous hydrogen peroxide, polyphenol compounds, 1,2-dicarbonyl compounds, and bee defensin-1 appear to be components that contribute to honey's antimicrobial activity (20). The synergic action of these compounds allows honey to be active against various types of microorganisms, including multidrug-resistant bacteria (1, 19). In addition, manuka honey, a monofloral honey obtained from Leptospermum scoparium, contains high concentrations of methylglyoxal (MGO), an organic compound with an antimicrobial activity whose concentration defines the unique manuka factor (4, 6). Probably this factor determines a higher bacterial activity of manuka honey compared to other types of honey. And yet, manuka honey has received more attention in the West due to its antimicrobial activity, whereas Arabs believe Yemeni Mountain Sidr honey (Ziziphus spina-christi) is superior (17). It remains to be investigated which honey has superior effectiveness and what is its mechanism of action.

Materials and methods

The study was performed on one commercial honey, manuka honey (MH), with MGO 500, produced by Auribee, New Zeeland. It was prepared an alcoholic extract using 1 g of honey, over which 10 ml of 70% ethanol was added. The samples were stirred with a hot stirrer plate (IDL, Freising, Germany) for 30 minutes. After stirring, the samples were filtered through filter paper.

The strains were isolated from dog otic secretions and purulent skin lesions and confirmed by API 20 Staph and API 20NE. From all the studied cases, 11 strains of *S. aureus* and 18 strains of *P. aeruginosa* were isolated.

The antimicrobial activity of honey was tested by the microdilution method (Fig. 1.).

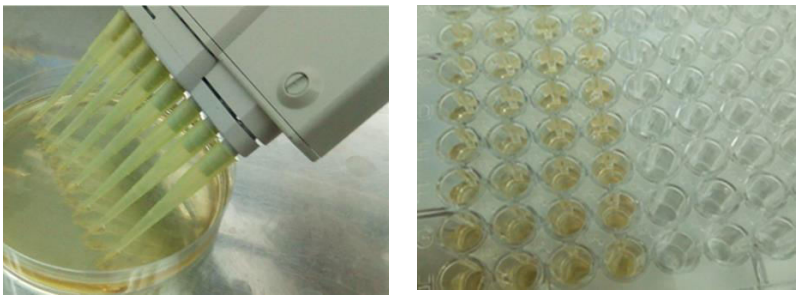

Fig. 1. The microdilution methods

A 10^{-3} dilution of each culture (on BHI broth) was used to perform the assay, an inoculum equivalent to a 0.5 McFarland standard. The suspensions were tested by spotting 100 µL of microbial suspension in each well of the 96 microdilutions well plate, using a Calibra digital 852 multichannel pipettes. The alcholic extract honey samples were added into wells at a concentration of 10%, 15% and 20%. The plates were covered and incubated for 24 hours at 37 °C, in aerobic condition. After 24 hours the OD was measured at 540 nm using an ELISA reader (BIORAD PR 1100, Hercules, CA, USA). Triplicate tests were performed for all samples.

Results and discussions

The obtained results, after testing different concentrations (10%, 15% and 20%) of manuka honey against *S. aureus* isolated strains from otic secretion and, pyogenic lesions of the skin, are presented in figure 2.

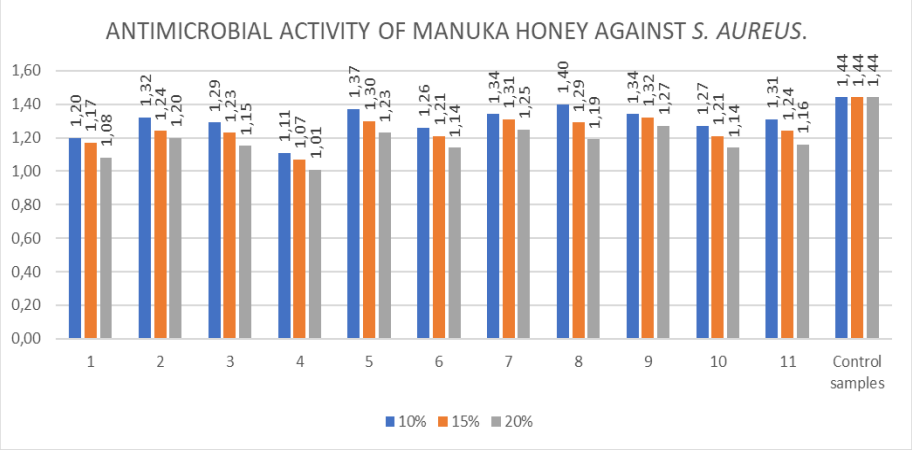

Fig. 2. Antimicrobial activity of manuka honey against *S. aureus*, expressed by O.D.

At a concentration of 10% of manuka honey, the O.D. varied between 1.11 and 1.40, the lowest value being obtained for strain four and the highest for strain eight of *S. aureus*. At a concentration of 15%, the O.D. values were between 1.07 for strain four and 1.32 for strain seven of *S. aures*. The O.D. values at a concentration of 20% of manuka honey were between 1.01 (strain four of *S. aureus*) and 1.27 (strain nine of *S. aureus*). The O.D. values for all the bacterial cultures with different manuka honey concentrations were lower than the control samples (1.44). This fact suggests that the minimum inhibitory concentration for all strains of S. aureus studied is 10%.

The results obtained by studying the different concentrations of

manuka honey against *P. aeruginosa* are presented in Fig. 3.

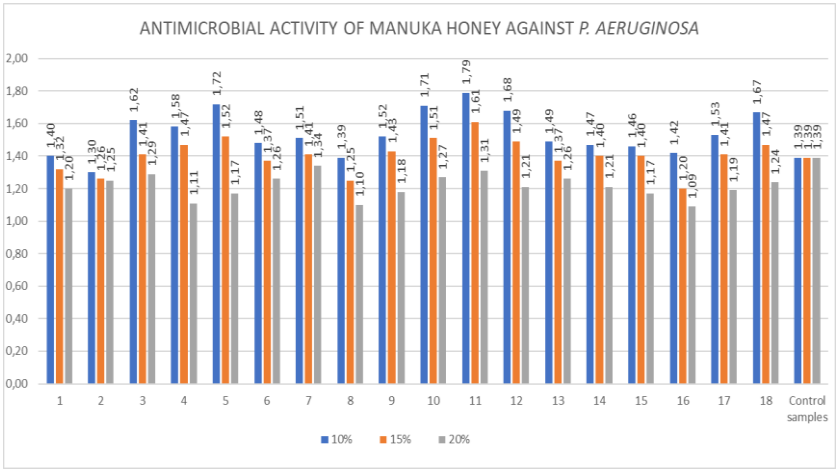

Fig. 3. Antimicrobial activity of manuka honey against *P. aeruginosa*, expressed by O.D.

As it is seen in figure 2, only strain two of *P. aeruginosa* is inhibited in the presence of 10% of manuka honey, with a value of O.D. of 1.20. A concentration of 15% manuka honey demonstrated antibacterial activity against six *P. aeruginosa* bacterial strains, respectively, q1cx against strains one, two, six, eight, thirteen and sixteen. The O.D. values for the strains sensitive to the concentration of 15% manuka honey were between 1.20-1.37. For the rest of the strains which were not sensitive to this concentration, the values of O.D. were between 1.41-1.61. The concentration of 20% manuka honey determined values of O.D. lower than the value of the control samples (1.39) for all *P. aeruginosa* tested.

Of all types of honey, manuka honey seems to be the most extensively investigated variety due to its bactericidal activity compared to others (3, 5, 7, 10, 12). Even though MGO seems to be the major factor in the antimicrobial activity of manuka honey, lower UMF (5+, 10+) showed antimicrobial activity comparable to the higher UMF (20+, 40+) (9). The MIC values of manuka honey vary depending on the bacteria group (11, 12, 13). Grecka et al., 2018, demonstrated that the MIC value for *S. aureus* was 3.125% (v/v), and for *P. aeruginosa* the MIC value was higher (6.25% v/v) (11). Other studies demonstrated that a MIC value of 22.5% has an inhibitory effect against both *S. aureus* and *P. aeruginosa* strains (5). Similarly, the present study indicates that a concentration of 10% is sufficient to inhibit *S. aureus* strains, while the MIC for all *P. aeruginosa* strains is 20%. In contrast, Bouzo et al., 2020, highlighted that the MIC value for *P. aeruginosa* is lower, respectively 10% (2). Moreover, other studies demonstrated wide MIC limits for S. aureus, between 3.11%

- 25% (7, 10, 11). All these data suggest that the MIC value of manuka honey is dependent from one strain to another, so further investigations are needed to establish the dose necessary to inhibit bacterial growth.

Conclusion

Testing the antimicrobial activity of manuka honey, MGO 500, by the microdilution method, it was concluded that manuka honey could be an alternative treatment for infections caused by pyogenic bacteria. However, MIC values are different, depending on the species involved in the pathology. For *S. aures*, the MIC value is 10%, while for *P. aeruginosa* is higher than that. A concentration of 20% of manuka honey is active against all the *P. aeruginosa* strains tested in the present study.

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CONGLUTINATING BOVINE SERUM AS A LIMITING FACTOR IN SEROLOGICAL REACTIONS

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Summary

Saiduldin Test use conglutinating bovine serum, which contains complement and conglutinin, as a limiting factor. This test was used by us to detect anti-brucellosis antibodies in the blood serum of camels. Comparative studies with conventional serological tests have shown a high sensitivity of ST in the diagnosis of brucellosis in camels.

Keywords: Saiduldin test, conglutinating serum, antibodies, brucellosis, camel.

The manifestation of serological reactions as a phenomenon based on the properties of immune serums was explained by J. Bordet in 1885. Therefore, the formation of serology proceeded as the formation of the science of the properties of immune serum chronologically during a period of heated disputes between supporters of the cellular theory of immunity and the humoral theory of immunity put forward by Ehrlich and Sachs (1, 5).

The study of the immune properties of blood serum made it possible to establish such serological phenomena as bactericidal and bacteriostatic properties, neutralization of bacterial exotoxins, the results of which were visually manifested in the form of crowding with the formation of large flakes (agglutination) or turbidity when mixing transparent components (precipitation). Whole cells under the action of immune serum were subjected to lysis (erythrocyte hemolysis, microbial cell bacteriolysis). The study of these phenomena made it possible to develop such classical diagnostic methods as the agglutination reaction (AR), precipitation reaction (PR) and complement fixation test (CFT).

The discovery of CFT was a major achievement of immunology, which determined the conduct of fundamental research and allowed a deeper insight into the essence of immunological reactions.

The CFT was proposed in 1901 by J. Bordet and O. Gengou (2). This was preceded by the discovery of complement in 1895 by J. Bordet, who established that erythrocytes and bacterial cells undergo lysis under the influence of specific thermostable and nonspecific thermolabile factors. The first of them is an antibody, and the second is called alexin, Erlich and Sachs – complement (2, 5).

The obligatory components of all serological reactions are antigens and antibodies, which are the main factors.

Some reactions also involve other components, such as complement, conglutinin, antiglobulin, anti-idiotype, which react with antigen-antibody complexes. These components are **additional reaction factors**.

Of these additional factors, complement and conglutinin, which functionally enter into serological reactions as a single conglutinating complex, determine the interaction of antigen-antibody complexes. If the interaction of an antigen with an antibody leads to the formation of visually detectable aggregates in the form of agglutination or precipitation without the participation of additional factors, then we are dealing with primary aggregation reactions.

If aggregation occurs with the participation of additional factors, then secondary aggregation reactions take place. In secondary aggregation reactions with the participation of a conglutinating complex as an additional factor, the antigen-antibody complex interacts with each other.

Therefore, conglutinating complement and conglutinin are of the most important importance for the detection of immune complexes from all additional factors of serological reactions (19).

It has been observed that fresh blood resists rotting better than other decomposable materials. This observation led to a number of studies to clarify the mechanism of action of fresh blood. Later it was found that the blood of intact animals has a bactericidal effect.

Bactericidal activity of the blood of healthy animals against anthrax bacilli was found. Later, the bactericidal effect of various fluids of the macroorganism was revealed.

It has been established that the bactericidal and lytic effect of immune serum is due to the presence of two substances: one of them increases during immunization, is specific to this type of microbes or cells, and is thermally stable (sensitizer); the second is contained in any fresh normal and immune serum, does not increase during immunization, is nonspecific, thermolabile, that disappears when heated to 56° (aleksin). The sensitizer is most often called lysine - bacteriolysin, hemolysin – depending on the antigen on which it has an effect. The phenomenon of conglutination was first described by Ehrlich and Sachs (5), Bordet and Gay (1), Muir and Browning (10), Bordet and Streng (5).

They found the presence of a special thermostable substance in the blood serum of cattle, which glued together red blood cells treated with antibodies and complement. It did not have such an effect on erythrocytes sensitized by antibodies in the absence of complement. This substance was initially called a bovine colloid, later – conglutinin, and the phenomenon of gluing was called conglutination (3).

Next, Streng O. (21) demonstrated conglutination using diphtheria, tuberculosis and *E coli* bacteria instead of erythrocytes.

After that, it became obvious that any antigen-antibody-complement complex, regardless of the specific origin of the components, can interact with conglutinin. Due to the specificity of this reaction, Streng O. (21) proposed using the phenomenon of conglutination as a diagnostic test and developed two variants of its

formulation: the direct conglutination reaction and the adsorption reaction of the conglutinating complement (22).

Currently, there are other varieties of the conglutination reaction: conglutinating complex fixation test (CCFT), conglutinating complex long fixation test (13), and immune complexes aggregation test - ICAT (15).

CCFT is based on the interaction of the antigen-antibody complex with the conglutinating serum, the latter being the limiting factor of the reaction (8, 9, 11, 12, 14, 18, 20).

For its production, the same equipment and components are used as for the CFT. The difference is that the guinea pig complement is replaced by bovine blood serum (conglutinating serum). The method is proposed for the detection of brucellosis antibodies in the blood serum of cattle. According to Saidouldin T. (13), in brucellosis of this animal species, CFT sensitivity exceeds the total results of AT, CFT, complement long fixation test (CLFT), and roz bengal test (RBT).

Currently, according to the instruction approved by the Veterinary Committee of the Ministry of Agriculture of the Republic of Kazakhstan, the test was called the Saidouldin Test - ST (17).

ICAT was developed based on the differences and properties of homologous and heterologous complexes. Homologous (consisting of one antigen and antibodies to it) immune complexes aggregated under the action of complement and conglutinin disintegrate under the influence of substances fixation calcium ions, whereas with the same effect heterologous complexes formed from two or more antigens and their corresponding antibodies are not homogenized (16).

The purpose of our research was a comparative test of the Saiduldin Test (ST), where the limiting factor is bovine conglutinating serum, with generally accepted tests for brucellosis in camels.

Materials and methods

In the experiments, 283 camel blood serum samples from camel breeding farms in Atyrau and Oral regions were used.

AT, CLFT and RBT for brucellosis were performed according to generally accepted methods (6, 7, 23). ST was set according to the existing instructions (17).

Results and discussions

The blood serums of 117 camels from unfavorable farm of Atyrau and Ural regions were studied. At the same time, all serum samples were titrated in each serological reaction to the limit titers of antibody.

The results of the studies are shown in Table 1. All 24 samples that reacted negatively to ST showed a negative result for both CLFT and AT.

Brucellosis was diagnosed by ST in 93 (14.42%) cases (n=117), whereas by CLFT – in 59 (9.15%) and by AT – 47 (7.29%), according to the total results of the

last two reactions in 59 cases, and ST+ LTCFT +AT in 93 cases.

Table 1

Results of ST in comparison with CLFT and AT with camel blood sera in brucellosis

ST		CLFT							AT				
Titres	**No**	**1:5 (-)**	**1:5**	**1:10**	**1:20**	**1:40**	**1:80**	**1:160 and higher**	**1:25 (-)**	**1:50**	**1:100**	**1:200**	**1:400 and higher**
1:10 (-)	24	24							24				
1:10	13	8	3	2					11	2			
1:20	7	6	1						6	1			
1:40	13	12		1					12	1			
1:80	8	3			4	1			7		1		
1:160	7	2			3	2			3	2	2		
1:320	15	2				7	4	2	3	2	4	6	
1:640	9	1				1	3	4	1		1	3	4
1:1280	8						1	7	2		1	1	4
1:2560	13							13	1				12
Total	**117**	**58**	**4**	**3**	**7**	**11**	**8**	**26**	**70**	**8**	**9**	**10**	**20**

Positive results of CLFT and AT in all cases are confirmed by positive results of ST. Of the 28 samples that reacted negatively in total according to CLFT and AT, 8 reacted positively in ST.

Thus, ST exceeds the total results of AT and CLFT by more than one and half times and is a sensitive test in the diagnosis of camel brucellosis.

The blood serum of 166 camels was studied in AT, CLFT, RBT and CFT (Table 1, Fig. 1).

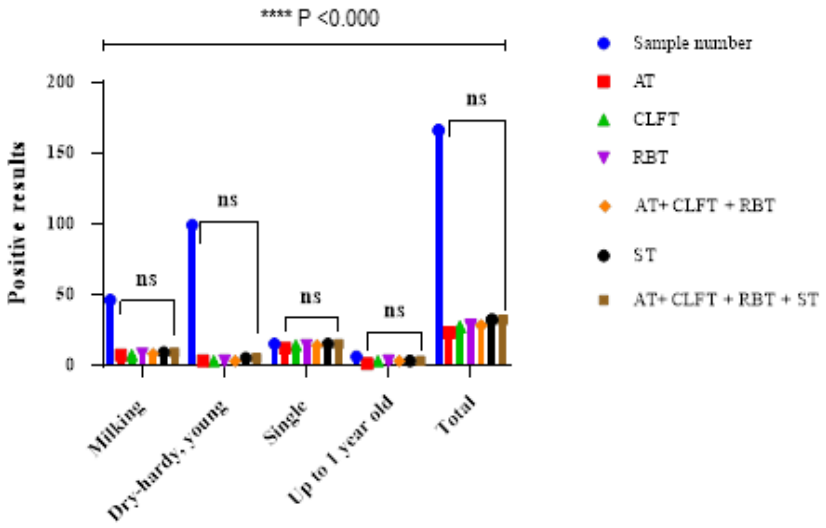

Fig. 1. Results of serological studies of camel blood serum for brucellosis
**** P <0.000; ns - the difference is not significant; the analysis was carried out by a two-way ANOVA followed by a Tukey multiple comparison test

Table 2

Results of serological studies of camel blood serum for brucellosis

Age and sex group of camels	Number of sample	Positive results					
		AT	CLFT	RBT	AT+ CLFT + RBT	ST	AT+ CLFT + RBT + ST
Milking camels	46	7	7	8	8	9	9
Dry-hardy camels and young animals	99	3	3	3	3	5	5
Single camels	15	12	14	14	14	15	15
Camels up to one-year-old	6	1	3	3	3	3	3
Total	**166**	**23**	**27**	**28**	**28**	**32**	**32**

The results presented in Table 2 and Fig. 1 show that 23 (13.9%) responded positively to AT, 27 (16.3%) to CLFT, 28 (16.9%) to RBT and 32 (19.3%) to ST. The average titers were in AT 1:110 (+6.4%; -6.0%), in CLFT – 1:27 (+20.6%; -17.1%), in ST – 1:65 (+15.7%; -13.5%).

The difference between the indicators of AT and CLFT, AT and ST was insignificant (P>0.05), and between CLFT and ST – significant, and reliable (P<0.05).

Thus, ST is a highly sensitive test for the diagnosis of camel brucellosis, the results of which exceed the total indicators of AT, CLFT and RBT.

Conclusions

The positive results of CLFT and AT complement each other.

All positive results of CLFT and AT are confirmed by similar indicators of ST.

The total indicators of CLFT, AT and RBT are confirmed by the positive results of ST.

ST is a highly sensitive test for diagnosing brucellosis in camels.

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CONTROL STRATEGY FOR SUBCLINICAL MASTITIS IN DAIRY COWS ON FARMS IN THE PORO REGION (CÔTE D'IVOIRE) IN 2022

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Summary

Subclinical mastitis represents the presence of infection of the mammary glands without visible signs of local inflammation or systemic involvement. The results of the analysis, concerning subclinical mastitis in dairy cows, after having investigated 45 farms in the Poro region out of a total of 360. The data were collected in four departments of the Poro region (Côte d'Ivoire) between May and August 2022. The samples consisted of fresh milk from the 4 quarters of the 288 cows with subclinical mastitis. Using the CMT test (California Mastitis Test), cases of mastitis have been observed in the four departments of the Poro region: Korhogo, Sinématiali, Dikodougou, and M'bengué. The MIRAH (Ministry of Animal and Fishery Resources of Korhogo) was informed and milk samples were taken from each farm and transported to the LANADA laboratory for confirmation of infection. The infection was confirmed by laboratory examinations, showing the major species (*Staphylococcus lentus, Staphylococcus xylosus, Staphylococcus aureus,* and *E. coli*) implicated in the etiology of mastitis in the region, and was associated with an increase in somatic cells in the milk of each quarter of dairy cows affected. Until the end of the survey on 288 dairy cows, in the Poro region, the results were: 80% had a score greater than or equal to 2, and the remaining 20% had a score greater than or equal to 1. For lactation months, the study showed that there is an influence on the occurrence of disease after the CMT test in young cows of 76.39% positive cases and then increases to 100% for older cows. After establishing the diagnosis, subclinical mastitis control measures were taken, and farmers were advised to separate infected cows (288) from healthy cows and to treat them at the dry-off. Cows with a score greater than or equal to 4 (90 dairy cows) were treated with antibiotics daily until the infection was eradicated. From May 5, 2022, to August 2022, subclinical mastitis was detected on dairy farms in the Poro region. Farmers have been informed of the need to apply hygiene measures and quarantine infected cows to control mastitis. For all cows with a score of 4 (90 dairy cows), the measure is considered antibiotic treatment.

Keywords: subclinical mastitis, dairy cows, pathogens, Ivory Coast.

The safety of consumables is an increasingly important public health concern (16). Increasing the level of milk production and improving the hygienic quality of the milk produced is, among other things, one of the challenges faced by dairy cattle farms. These challenges cannot be met without better knowledge of the etiological agents involved in mastitis and by developing adequate control and prevention strategies (2).

Escherichia coli is an environmental pathogen, however, this bacterium can persist in the mammary gland, indicating that some *E. coli* will possess assets similar to those of contagious agents (8). However, the exact determination of the identity of the bacteria responsible for mastitis is just as essential as it is important in the orientation of the therapeutic choice, just as in the control of infection on a farm (2).

Regarding, mastitis control programs, milking hygiene is a crucial phase. Semmelweis' work on puerperal fever demonstrated that hand hygiene by health workers in charge of the delivery of pregnant women significantly reduced the mortality rate of women from puerperal fever (20).

Milk, considered a complete food, occupies more and more an important place in the daily diet of populations in the world in general and in sub-Saharan Africa in particular. Production is mainly from cows in traditional breeding, animal health care is done empirically, involving anarchic use of pharmaceuticals, including antibiotics.

Antibiotics are used in animal husbandry systems for prevention or as feed additives or growth promoters (21). This type of use induces changes in animals' digestive flora, leading to the emergence of resistant strains (11). Also, non-compliance with waiting times after treatment leads to antibiotic residues in animal products, including milk (17).

The emergence of antibiotic-resistant microorganisms has become a significant concern for the World Health Organization since the appearance of multi-resistant pathogenic strains for humans, causing treatment failures (10).

In West Africa, bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Salmonella enterica*, *Shigella spp*., original clinical, environmental and dietary will have proven to be increasingly resistant to commonly used antibiotics (3). In several African countries, given the technical means of detection, there is little data on the presence of antibiotic residues in foodstuffs (14). Non-compliance with hygiene conditions and the anarchic use of unsuitable antibiotic molecules have led to problems of resistance and the persistence of mastitis, especially clinical mastitis in dairy farms, hence the increase in the rate of cows reconstructed for uncured mastitis.

If the diagnosis of clinical mastitis is relatively easy thanks to the visible clinical signs, that of subclinical mastitis is less so. But, thanks to certain tests (CMT, CCS), screening for subclinical mastitis becomes useful (13).

Materials and methods

The study was carried out between May and August 2022 in 45 Poro region (Côte d'Ivoire) farms, examining 360 dairy cows. For each cow from the four departments of the Poro region included in the study, data were collected concerning age, the month of lactation, breed, the presence or absence of vaccination, some clinical signs of disease, and the treatments applied. Data were analyzed using Office Excel software.

The animals submitted to the study came from traditional farms, having different owners or associations. Some of the studied cows, non-vaccinated or fully vaccinated, presented relevant clinical signs specific to mastitis, with the presence of wounds on the teats, deformation of the quarters, or refusal to alert the calf (Fig. 1A and B).

Fig. 1 A. Wounds on the teats, B. Distortion of the quarters

For laboratory analysis, fresh milk samples were collected from the 4 quarters and sent to the LANADA laboratory (NATIONAL LABORATORY FOR AGRICULTURAL DEVELOPMENT SUPPORT) in Korhogo.

The California Mastitis Test (CMT) (Fig. 2) was used to estimate the somatic cell count of milk. The test result is not numerical but rather an indication of whether the cell count is high or low. The CMT shows changes in cell counts above approximately 400,000 cells/ml.

Fig. 2: Califormia Mastitis Test (CMT)

Based on the results of the CMT test, each cow was assigned a score from 0 to 4.

Results and discussions

The prevalence of mastitis assessed by CMT test after investigation of 360 milk samples is present in Fig. 3.

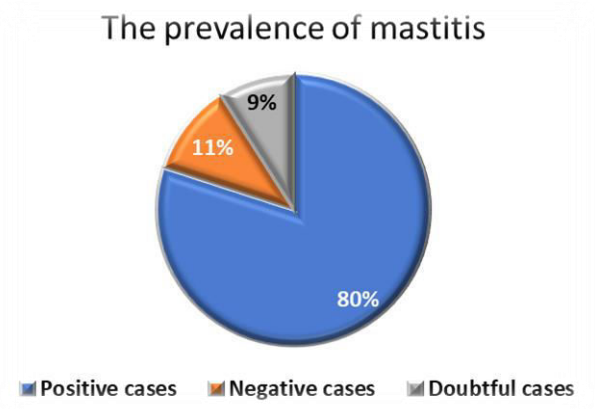

Fig. 3. The prevalence of mastitis by using the CMT test

The results of the CMT test are presented in Table 1.

Table 1

Prevalence of mastitis according to the breeds of cows

Scores	Mérés	N'dama	Baoule	Zebus	Half Breed	Total
0	38	-	2	-	-	40
1	30	2	-	-	-	32
2	12	-	-	2	-	14
3	164	12	2	2	4	184
4	78	6	4	2	-	90
TOTAL	**322**	**20**	**8**	**6**	**4**	360

As seen in Table 1, of the 360 dairy cows, 288 (80%) were positive for the CMT test with a score greater than or equal to 2, and 72 had a score equal to 0 and 1. The number of cows with a score equal to 4 was 90 dairy cows out of 288 positive ones.

The number and the percentage of positive cows in the age category are presented in Table 2.

Table 2

Prevalence of mastitis according to the ages of cows

Departments	Scores by CMT	AGES				Total	Prevalence %
		3-5	6-9	10-12	13-15		
KORHOGO	Negatives ≤ 1	8 (19.05%)	2 (5%)	0 (0%)	0 (0%)	10	10.42
	Positives ≥ 2	34 (80.95%)	38 (95%)	8 (100%)	6 (100%)	86	89.58
	Total	**42**	**40**	**8**	**6**	**96**	**100**
M'BENGUE	Negatives ≤ 1	0 (0%)	2 (3.03%)	0	0	2	2.08
	Positives ≥ 2	30 (100%)	64 (96.97%)	0	0	94	97.92
	Total	**30**	**66**	**0**	**0**	**96**	**100**
SENEMATIALI	Negatives ≤ 1	20 (47.61%)	22 (47.82%)	2 (25%)	0	44	45.83
	Positives ≥ 2	22 (52.38%)	24 (52.17%)	6 (75%)	0	52	54.17
	Total	**42**	**46**	**8**	**0**	**96**	**100**
DIKODOUGOU	Negatives ≤ 1	6 (20%)	8 (26.66%)	2 (25%)	0 (0%)	16	22.22
	Positives ≥ 2	24 (80%)	22 (73.33%)	6 (75%)	4 (100%)	56	77.78
	Total	**30**	**30**	**8**	**4**	**72**	**100**

In young cows (3-5 years), the disease was diagnosed at a percentage of 76.39%. The percentage of positive cases increases as the age increases from 81.32% in the 6-9 years category to 83.33% in the 10-12 years category. The percentage of positive cows for the age category over 12 years old was 100%. Baoulé and Zébus are more resistant than the others because the cows selected by humans for their milk production are predisposed to mastitis (7).

The overall prevalence of subclinical mastitis obtained in another study on two farms was 58.53% (13). This percentage remains high compared to those obtained in Senegal for half-breeds and by Bosquet (1) for local breeds in Niger, which are 46.2% and 44%, respectively. This high rate could be justified by the fact that the breeds selected by humans for their milk production are predisposed to mastitis (**7**). This observation is in accord with our study, which demonstrates a significant difference between the breeds of cows (Baoulé, Zebus, Mérés, N'dama) and half-breeds concerning the positive CMT test.

The analysis after mapping, concerning the mastitis detection zone, revealed an increased number of positive cases in the CMT test in the departments of Korhogo, Dikodougou and M'bengué compared to the department of Sinématiali in the Poro region (in northern Côte d'Ivoire).

The measures taken to reduce the case of bovine mastitis include the administration of antibiotics and practical rules of hygiene. Antibiotic treatment lasts 5 to 21 days for severe cases, using the first-line antibiotic. If, after the treatment,

the results of the test CMT were positive again, bacteriological examination and antibiograms were recommended (6**).**

Some studies show that mastitis infection is mostly symptomatic and asymptomatic. And that the treatment proposed by the veterinary practitioner is based on the herd's epidemiological model established from the breeding documents and a bacteriological survey (18). Subclinical mastitis is treated at the drying off, with rare exceptions, during lactation. The cure rate for subclinical mastitis during lactation is 50% on average compared to 70 to 80% at drying off (6).

The treatment for the cows with a score of 4 consisted of administering prilimycin, gentamicin, and cloxacillin by the intramammary route or penethamate by the general route.

Regarding the age category, our study showed a correlation between age and the disease's appearance. Using the CMT test, the percentage of positive cows was 76.39% in the young category and fluctuated up to 100% for older cows. This result agrees with other studies (9). The increased cell count in older cows may be linked to the decline in natural defenses in the mammary gland (9). Increased cell number noted at the end of lactation could be explained by increased cell concentration in a small volume of milk or by contamination of the animals during or between the drafts. Thus, the susceptibility of the udder to infection increases with the number of lactations (7). Highly positive data can therefore be obtained with older dairy cows or cows at the end of lactation.

Conclusions

From May to August 2022, subclinical mastitis was detected in dairy farms in the Poro region. Farmers have been informed of the need to apply hygiene measures and treat cows that were positive for the CMT test, to fight against mastitis. The cows with a score of 4 (90 dairy cows) were treated with antibiotics.

Acknowledgment

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PARACLINICAL CHANGES OCCURRING IN FELINE HYPERTHYROIDISM

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Summary

Feline hyperthyroidism is an endocrine disease caused by hypersecretion of thyroid hormones, being the most common endocrinopathy found in cats. With its onset, a series of associated paraclinical alterations appear. The aim of the study was to assess the diagnostic value of the hematological and biochemical changes in feline hyperthyroidism. The study was performed on 15 cats diagnosed with hyperthyroidism, which attended the Internal Medicine Clinic of the Faculty of Veterinary Medicine in Timisoara. For this study, the results of blood tests from the time of diagnosis of hyperthyroidism were analyzed. The complete blood count was determined by flow cytometry and the blood biochemical parameters, respectively aspartate aminotransferase (AST/GOT), alanine aminotransferase (ALT/GPT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), urea, creatinine, albumin and total proteins, were determined from the blood serum by usual methods. The biochemical profile revealed a higher magnitude increase in mean ALT and ALP values, at 93% and respectively 80% of the cases studied. Serum AST and GGT activity showed small magnitude increases in only 73% and respectively 20% of cats diagnosed with hyperthyroidism. The complete blood count showed no clinically significant changes in the cats in this study. The magnitude of thyroid hormone elevation correlated positively and significantly with serum ALP and ALT activity. Hyperthyroidism should be considered in any older cat that exhibits polyphagia, weight loss and increased serum ALT and ALP activity.

Keywords: cat, hyperthyroidism, biochemical parameters.

Hyperthyroidism is an endocrine disease characterized by an excessive production and secretion of thyroid hormones (12,19). Hyperthyroidism is the most common endocrinopathy found in cats (6, 14). If untreated early, an overactive thyroid can be life-threatening (1). Given the multifactorial etiology of this condition, as well as the difficulty of confirming a diagnosis, numerous procedures have been proposed over the years to evaluate thyroid function in cats suspected of hyperthyroidism to confirm or rule out the diagnosis, including clinical examination, blood pressure measurement, imaging diagnostic, scintigraphy, and laboratory testing (4, 10, 16).

The biochemical profile reflects abnormal but non-specific values of hyperthyroidism and may include changes in the following parameters: increased alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP). Although these findings are non-specific, they are useful to confirm or rule out concurrent diseases or to make differential diagnoses (8, 9).

Materials and methods

The study was performed on 15 cats diagnosed with hyperthyroidism, which attended the Internal Medicine Clinic of the Faculty of Veterinary Medicine in Timisoara. For this study, the results of blood tests from the time of diagnosis of hyperthyroidism were analyzed.

Blood biochemistry and complete blood count (CBC) analyses were performed in the Laboratory of Metabolic and Functional Explorations from University of Life Sciences "King Mihai I of Romania" Timisoara. The CBC was determined by flow cytometry using an automatic hematology analyzer: IDEEX-ProCyte Dx. Blood biochemical parameters were determined from blood serum by standard methods with an automated biochemistry analyzer: Randox- Rx Daytona+.

From all cats, blood samples were collected in the morning following a 12-hour fasting period and were collected from the jugular vein in vacuum tubes containing EDTA K3 for CBC determination and in simple or gel tubes for blood biochemical examination. Serum was separated from the erythrocyte mass within approximately 2 hours of sample collection and refrigerated until analyzed.

Serum total thyroxine (T4) and thyroid stimulating hormone (TSH) were determined by chemiluminescence methods.

Individual values of biochemical parameters were statistically processed and are presented in the paper as mean ± standard deviation. Statistical analysis was performed using the computer program SPSS (Statistical Products of Services Solution), version 20.

Results and discussions

The mean values of blood biochemical parameters investigated in cats with hyperthyroidism in this study are shown in Table 1.

A moderate but significant correlation ($p<0.05$) between thyroid hormone (T4) concentration and serum ALP and ALT activity was found in cats in this study.

The biochemical profile reveals an increase of a higher magnitude in the mean values of ALT and ALP. Thus, the mean value of ALT was 275.5±137 U/L (Fig. 1a), with the serum activity of this enzyme showing significant increases over baseline values in 93% of the cases studied (Fig. 1b).

Table 1

Descriptive statistics of blood biochemical parameters in cats with hyperthyroidism

Biochemical parameters	MU	Mean	Standard deviation	Standard Error	Reference Range
AST/GOT	U/L	66.17	6.52	15.98	10-50
ALT/GPT	U/L	275.58	39.57	137.09	10-70
GGT	U/L	4.22	0.68	2.05	0-6
ALP	U/L	138.25	17.83	61.77	35-120
Creatinine	mg/dL	1.24	0.20	0.70	0,9-1,7
Urea	mg/dL	84.93	17.55	52.66	7-28
Total protein	g/dL	6.80	0.13	0.35	5,4-7,4
Albumin	g/dL	3.20	0.19	0.46	2,7-4,7
T4	mg/dl	9,59	3,93	1,18	1-4

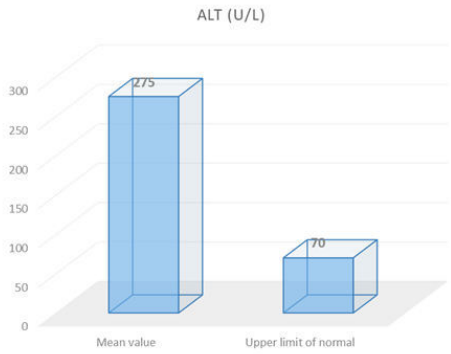

Fig. 1a. Mean values of serum ALT activity between cats with hyperthyroidism and reference range

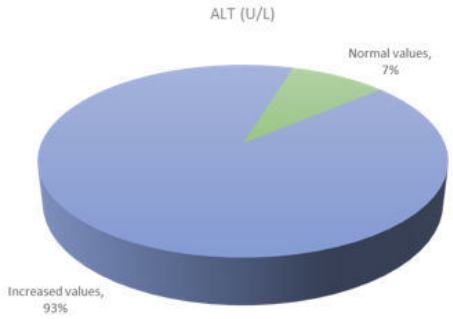

Fig. 1b. Prevalence of increased serum ALT activity in cats with hyperthyroidism

Serum alkaline phosphatase activity (ALP) recorded a mean value of 138.25 U/L ± 17.8 U, which was above the upper limit of the reference values (Fig. 2a), and in 80% of cases it exceeded the upper limit of the reference values (Fig. 2b.)

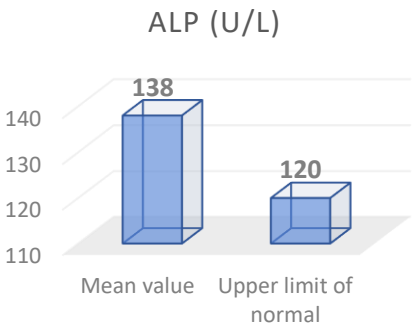

Fig. 2a. Mean values of serum ALP activity between cats with hyperthyroidism and reference range

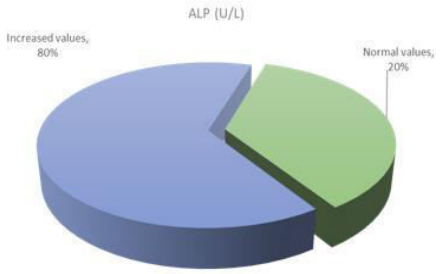

Fig. 2b. Prevalence of increased serum ALP activity in cats with hyperthyroidism

In other studies, the most common biochemical changes consisted of increased serum ALT and ALP concentrations, which were reported in >90% of hyperthyroid cats (13, 14).

Although in most cases the increases in ALP and ALT serum activity are of medium magnitude, sometimes increases of very high magnitude can also be observed. There is little data on the mechanism that causes liver enzyme increases in cats with hyperthyroidism. In contrast, in hyperthyroid rats, a direct toxic effect of T3 has been observed resulting in lipid peroxidation, protein oxidation, glutathione depletion in liver mitochondria and subsequent apoptosis (3). Some feline studies have shown that both hepatic and bone ALP isoenzymes contribute to serum ALP elevation in hyperthyroid cats (7, 15, 16). Although previous recommendations included evaluation of hyperthyroid cats with ALP or ALT activity >500 IU/L for detection of concomitant disease, subsequent studies have shown that changes seen in the liver secondary to hyperthyroidism do not usually influence liver function tests such as pre- and postprandial serum bile acids, so such testing may not be warranted (5). In addition, histological assessment of the liver in cats with hyperthyroidism reveals nonspecific and moderate changes, and liver enzyme changes associated with hyperthyroidism usually return to baseline values with successful treatment of the disease (5, 13).

Although ALT and/or ALP elevations are usually mild, severe elevations may be seen. In one study, a positive and significant correlation was found between T4 concentrations and serum ALP activity (11). Normal concentrations of these enzymes in cats with an enlarged and palpable thyroid gland, but with a normal T4 concentration, could be an argument supporting the euthyroid status of these animals (14)

Gamma-glutamyltransferase (GGT) recorded a value of 4.2±0.6 U/L, the limits of variation being between 0-7 U/L (Fig. 3a). In contrast to alkaline phosphatase, serum GGT activity was within normal limits in 80% of cases and exceeded the reference values in only 20% of the cats (Fig. 3b). As an enzyme secreted mainly by the liver, the increase in serum activity of GGT can be attributed to concomitant liver damage.

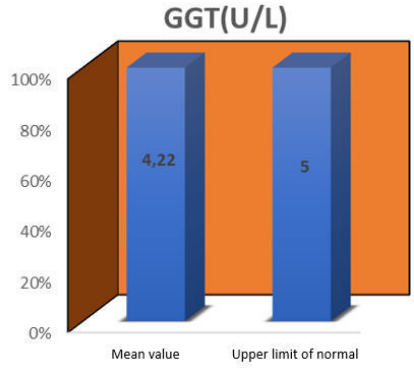

Fig. 3a. Mean values of serum GGT activity between cats with hyperthyroidism and reference range

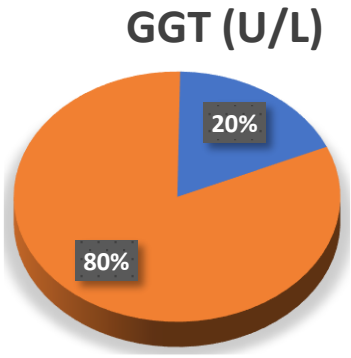

Fig. 3b. Prevalence of increased serum GGT activity in cats with hyperthyroidism

Serum creatinine concentration recorded a mean value of 1.2±0.2 mg/dL and individual values ranging from 1-3 mg/dL. The mean value of serum urea (BUN) was 84 ± 17.5 and 63% of cats recorded higher values than the physiological limit, termed in the specialty literature as azotemia In hyperthyroidism, azotemia is frequently reported because weakness, vomiting and dehydration are predominant clinical signs in hyperthyroidism (18, 20).

Erythrocytes recorded values between 5 and 10 mil/µL, with an average of 8.6 mil/µL, and haemoglobin showed an average value of 11.52 and values between 7-14 g%. Hematocrit recorded a mean value of 34.3% with individual variances ranging from 24.5%. Platelets recorded a mean value of 3.4 thousand/µL with mean values ranging from 2 to 5 thousand/µL. Leukocytes had a mean value of 8.6 thousand/µL with variations ranging from 5 to 14 thousand/µL.

Previous studies have found that about 40-50% of cats with hyperthyroidism have mild increases in haematocrit and about 20% develop macrocytosis. The erythrocyte changes are thought to be the result of increased erythropoietin in hyperthyroidism as well as a direct effect on the bone marrow through β-adrenergic stimulation mediated by thyroid hormones to increase red blood cell production and release (11). Common leukogram changes include neutrophilia, lymphopenia, eosinopenia and monocytosis (5, 13).

Conclusions

The increases magnitude of thyroid hormone concentration correlates positively and significantly with serum ALP and ALT activity.

Hyperthyroidism should be considered in any older cat with polyphagia, weight loss and increased serum ALT and ALP activity.

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EPIZOOTOLOGICAL SITUATION IN KAZAKHSTAN WITH REGARD TO ANIMAL LISTERIOSIS

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Summary

Analysis of statistical data of the veterinary reporting of the Committee for Veterinary Control and Supervision of the Ministry of Agriculture of the Republic of Kazakhstan for 2012-2021 shows the presence of listeriosis outbreaks in the territory of the country. During these years listeriosis was detected in Akmola, Almaty, Atyrau, Aktobe, Karaganda, Mangistau, and Turkestan regions. The most unfavorable regions for listeriosis incidence are Karaganda, Akmola, Aktobe, and Turkestan regions. In the Karaganda region, bovine listeriosis was registered annually from 2018 to 2021 in the Ulytau district, as well as in farms near Zhezkazgan city. In Akmola oblast, Bersuat rural district, Zhana Bereke LLP from April to May 2020, 7 cases were registered among sheep. According to veterinary department statistics in Aktobe oblast from 2012 to 2021, the disease in cattle and sheep was registered in Alginsk and Murgalzhinsk districts. In the Mangistau region by ELISA 3.3% of sheep, serum was positive; in the Aktobe region 2.8% of cattle and 2.9% of sheep samples were positive; in the Almaty region 4.4% of cattle serum and 2.1% of sheep tested positive; in Akmola region, 3.09% of cattle and 0.5% of sheep tested positive by ELISA. In the Karaganda region, 13.5% of cattle and 15% of sheep serum had antibodies to listeriosis at diagnostic titer; in the Zhambyl region, 8% of cattle and 8.3% of sheep were seropositive to listeriosis.
Keywords: listeriosis, cattle, epizootic process, antibodies, antigen.

Listeriosis is a zooanthroponotic disease of animals and humans, characterized by damage to the nervous system, septicemia, abortion, and mastitis (1, 2).

Listeria is widely distributed in the environment throughout the world. They are isolated from soil and aquatic ecosystems, from the external environment, circulate in wild, domestic, and farm animals, and cause development in them and humans (3, 4, 5). In this context, it is only logical that in the last decade close attention has been paid to listeriosis infection (6).

Listeria is relatively resistant and widespread in the external environment. The microorganism persists and multiplies well at low temperatures of 4-6ºC ("refrigerator microbe"). The listeriosis pathogen can survive in soil, water, straw, and grain for a long time up to several years (7).

It is known, that the most frequent and dangerous sources of the pathogen are sick domestic and wild animals with the acute course of listeriosis and bacterial carriers, especially rodents. Listeriosis transmission lasts about 30 days in cows, 300 days in cattle, and 260 days in some rodent species. Some cases of listeriosis in

animals are septic and lethal. Among animals under natural conditions, the pathogen is transmitted mainly by the alimentary route. Listeria is excreted from sick animals in all body secretions, such as faeces, urine, milk, saliva, and nasal secretions (8, 9, 10). They infect pastures, premises, water sources, feed, and foodstuff with their secretions. Once in the external environment, the listeriosis pathogen can survive for a long time, posing a threat of over-infection to animals. Animal buildings such as stables, stables, pigsties, and yards often harbor large numbers of rats and mice if they are not regularly exterminated. By roaming in manure, in pits with animal carcasses, and settling all over the premises, they contaminate feeders, fodder, and water, moving from one farm to another (11, 12, 13).

However, until now in Kazakhstan, neither the true level of listeriosis incidence nor its structure and dynamics are clear (14, 15, 16).

Since animal listeriosis can also be transmitted to humans, forecasting the occurrence of new cases of this disease is an urgent and necessary task (17).

The development of forecasting the incidence of animal listeriosis is possible only after a comprehensive study of the epizootological situation since this infection is a natural focal one. For example, confinement to a certain area of synanthropic rodents, bacterial carriage, natural-climatic conditions, etc. An important element of epizootological surveillance in the study of natural-economic conditions is the initial element of epizootological forecasting of the development of the epizootic process of many contagious diseases of animals, including listeriosis (18, 19, 20).

Materials and methods

Epizootological monitoring of farm animals in Kazakhstan for animal listeriosis was carried out by analyzing statistical data from veterinary reports of the Veterinary Inspection of the Ministry of Agriculture of Kazakhstan, as well as from the results of own studies during visits to farms in Kazakhstan. In addition, a schedule of scheduled visits to epizootological monitoring points was planned to conduct in-house investigations in order to inspect livestock for clinical diagnosis and take samples for laboratory diagnosis. For animal listeriosis monitoring we used the enzyme immunoassay method with standard test systems (ELISA).

Immunoenzyme analysis was made in "Omicron ZD" LLP, there was used a test system "Test kit for detection of individual-specific antibodies of class G to Listeria mocytogenes bacteria in blood serum (plasma) of agricultural animals (big and small cattle, pigs, horses, camels) by immunoenzyme method" (Sibbiotest NPF LLC).

The samples were taken for investigations in those regions of the country where outbreaks of listeriosis of animals were noted, i.e. Mangistau, Aktobe, Almaty, Akmola, Karaganda, Zhambyl regions.

To obtain a sample from each oblast 3 districts were planned to be selected, and from each district, 3 settlements were to be selected. 30 cattle and sheep serum samples were planned to be collected from each settlement.

Results and discussions

Analysis of veterinary reporting statistics from veterinary reports of the Veterinary Inspection of the Ministry of Agriculture of Kazakhstan for 2012-2021 shows the presence of listeriosis outbreaks in the territory of the country. During these years listeriosis was detected:

1. Akmola region - 4 outbreaks in sheep in 2020;
2. Almaty region - 1 outbreak in sheep in 2019;
3. Atyrau region- 1 outbreak in cattle in 2015;
4. Aktobe region - 2 outbreaks in sheep in 2012 and 2014 and 1 outbreak in cattle in 2021;
5. Karaganda region - 5 outbreaks in cattle from 2018 to 2021;
6. Mangistau region - 1 outbreak in cattle in 2016;
7. Turkestan region - 2 outbreaks in sheep in 2021;

The highest number of outbreaks of listeriosis pathogen was recorded in Karaganda, Akmola, Aktobe, and Turkestan regions; in addition, there are reports from private veterinary specialists about outbreaks in Mangistau, Atyrau and Almaty regions. In all cases, restrictive measures have been imposed by the local veterinary authorities and animal health procedures have been carried out.

In Karaganda Oblast, cattle listeriosis has been reported annually from 2018 to 2021 in the Ulytau district, as well as in farms near the city of Zhezkazgan.

In Akmola oblast, Bersuatskiy rural district, Zhana Bereke LLP, 7 cases of the disease were registered among sheep from April to May 2020. Clinical signs of the disease were characterized by lesions of the nervous system (profuse salivation, vertachka) and blood and internal organs of the fallen animals were delivered to the national veterinary laboratory. As a result of bacteriological tests and PCR analysis, listeria was detected, after which by decision of the Chief State Veterinary and Sanitary Inspector of Arshali district restrictive measures on listeriosis of small ruminants were taken in the farm.

According to statistics from the Veterinary Department of Aktobe oblast from 2012 to 2021 diseases of cattle and sheep were registered in Alginsk and Murgalzhinsk districts.

One of the last reported outbreaks of listeriosis in Kazakhstan was in Turkestan Oblast. The first outbreak was registered in Saryagash rayon, Kurkeles rural district, Algabas village, in MHP. The second outbreak was reported in Zhetisai district, rural district J. Eraliev, village Urlytobe, also in sheep.

Considering that materials from diseased and fallen farm animals are not always delivered for testing in national laboratories, we believe that statistical data of veterinary reporting does not reflect the objective epizootic situation of animal listeriosis. Thus, the remaining regions of the country are currently regions with unknown disease status, since no full-fledged monitoring studies on animal listeriosis have been conducted in them, which means that it is impossible to prove their welfare status or to confirm the presence of the pathogen.

To analyze the current epizootological situation of the territory of the country for animal listeriosis we carried out monitoring studies using an enzyme immunoassay.

Blood serum sampling was conducted in three districts of the Mangistau region: Mangistau, Munailey, and Tupkaragan districts. ELISA results showed that 4 (3.3%) out of 121 sheep blood samples were positive.

In the Aktobe region, blood samples were obtained in four districts - Alginskiy, Mugalzharskiy, Kobdinskiy, and Uilskiy. A total of 420 cattle blood samples and 416 sheep blood samples have been tested. Out of 420 cattle blood samples, 12 (2.8%) tested positive. Out of 416 samples from sheep 14 (2.9%) were positive.

In the Almaty region, blood serum sampling was conducted in three districts: Alakol, Enbekshikazakh, and Uygur. ELISA results showed that of 274 blood samples from cattle 12 (4.4%) were positive for ELISA. Out of 236 sheep blood samples, 5 samples were positive (2.1 %). It should be noted that all blood sera (105 cattle blood samples and 74 sheep blood samples) received from the Alakol region have negative results.

In Akmola oblast, Burabay, Arshalin, and Ereymentau districts were selected for the study. Three rural districts and three settlements were selected from each district. In total blood sera from 550 cattle, and 180 sheep were obtained for testing. By results of the ELISA test 17 (3.09 %) heads of cattle had antibodies to listeriosis in titer higher than 350 that is positive, and 18 (3.3 %) blood samples in the range 301-350. Out of 180 sheep blood samples, 1 (0.5%) was positive.

In the Karaganda region, 260 samples from cattle and 260 from sheep were collected for investigations. Samples were obtained from animals from Abay, Ulytau, and Nurin districts. By ELISA results 35 (13,5%) samples from cattle were found antibodies to listeriosis in diagnostic titer. Out of 260 blood samples from sheep 39 (15%) were positive.

In the Zhambyl region, there were 250 blood samples from cattle and 265 blood samples from sheep in three districts. 20 (8%) samples from cattle were seropositive for listeriosis. From the blood serum obtained from sheep 22(8.3%) were positive.

Conclusions

Thus, in the studied regions of the republic (Mangystau, Aktobe, Almaty, Akmola, Karaganda, and Zhambyl regions) there are agricultural animals seropositive to listeriosis.

Taking into consideration outbursts of listeriosis for the last 10 years and the results of our investigations, we have reasons to suppose, that there is a tense situation with listeriosis of agricultural animals in the country.

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STUDY ON THE PREVALENCE AND LARVAL BURDEN OF THE NEMATODE *TRICHINELLA SPP.* IN JACKALS FROM HUNTING GROUNDS IN TIMIS COUNTY (ROMANIA)

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Summary

Nematodes of *Trichinella* genus are cosmopolitan zoonotic parasites and are among the most widespread parasites of domestic and wild omnivores and predatory animals. The risk of infection with *Trichinella spp.* is a major concern in Romania due to eating habits. This disease is and remains one of the most important parasitic zoonoses, transmitted through the consumption of raw meat from domestic and wild animals. In the context that the jackals represent a sentinel of the intersection of the two domestic and sylvatic cycles, we followed this study to evaluate the prevalence and the larval distribution of *Trichinella spp*. in the muscle samples collected from jackals from Timis County (Romania) hunting grounds. The study was carried out during 2019-2021. The muscle samples from 42 jackals were examined by trichinelloscopy and artificial digestion. The results revealed a 78.57% prevalence of trichinelosis. The levels of larval distribution showed the high larval burden in the tongue muscles (37.8 larvae/gram), diaphragm (21.3 larvae/gram), intercostal (11.2 larvae/gram) and the lowest percentage was in the temporal muscle (3.25 larvae/gram). We can conclude that the high prevalence of trichinellosis in a sentinel host such as the jackal had a predominant and similar larval distribution to the one identified in domestic animals. The results of the present study warn about the risk of infestation with a possible zoonotic parasite identified in a host involved in the wild and domestic relationship with trichinellosis.

Keywords: jackal, trichinellosis, prevalence, larval distribution, Romania.

The golden jackal (*Canis aureus*) is one of the most widespread canid species with a range covering areas of central, eastern, and southern Europe, northern Africa, and parts of Asia. The epidemiological importance attributed to this species is relevant when the jackal plays the role of host for some parasites, with zoonotic character (1).

Nematodes of the *Trichinella* genus are cosmopolitan zoonotic parasites and are among the most widespread parasites of domestic and wild omnivores and predatory animals. The risk of infection with *Trichinella spp.* is a major concern in Romania due to eating habits (3, 9, 13).

This disease is and remains one of the most important parasitic zoonoses, transmitted through the consumption of raw meat from domestic and wild animals (5, 15, 18).

In this context, the purpose of this study was to evaluate the prevalence and the larval distribution of *Trichinella spp*. in the muscle samples collected from jackals from Timis County (Romania) hunting grounds.

Materials and methods

Muscle samples were collected from a number of 42 jackals hunted over three years (2019-2021) from hunting grounds in Timis County.

Trichinelloscopy and artificial digestion have been performed (8, 10).

The muscles examined were:

- Tongue muscles
- Head muscles (m. masseter/ m. temporal)
- Diaphragm
- Intercostal muscles
- Neck muscles (cutaneous neck muscles / dorsal and ventral cervical muscles)
- Spine muscles (m. great dorsal / m. thoracic trapezius)
- Muscles of the right / left thoracic limb
- Muscles on the left/right pelvic limb

Results and discussions

The results obtained by trichinelloscopy (Fig. 1, Fig. 2) and artificial digestion (Fig. 3) revealed a prevalence of 78.57% (33/42) in jackals from hunting grounds in Timis County.

Fig. 1. *Trichinella spp*. – tongue muscle

Fig. 2. *Trichinella spp.* – temporal muscle

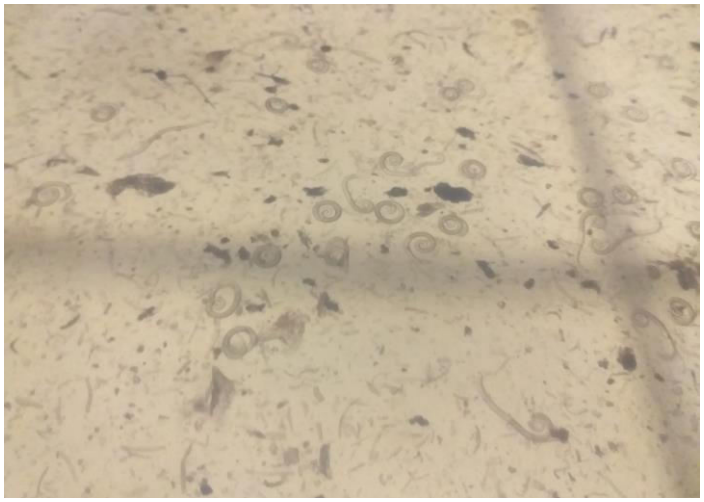

Fig. 3. *Trichinella spp.* – larvae

The distribution of *Trichinella spp*. larvae in the muscle samples were as follows:

- Tongue - 37.8 larvae/gram
- Masseter - 10.8 larvae/gram
- Temporal muscles - 3.25 larvae/gram
- Diaphragm - 21.3 larvae/gram
- Intercostal muscles - 11.2 larvae/gram
- Neck muscles - 8.7 larvae/gram
- ***Spine muscles***

- m. great dorsal – 8.32 larvae/gram
- m. thoracic trapezius – 9.89 larvae/gram

- ***Muscles of the right thoracic limb***

- Arm muscles - 10.31 larvae/gram
- Forearm muscles - 12.34 larvae/gram
- Shoulder muscles - 10.84 larvae/gram

- ***Muscles of the left thoracic limb***

- Arm muscles - 14.78 larvae/gram
- Forearm muscles - 8.56 larvae/gram
- Shoulder muscles - 8.23 larvae/gram

- ***Muscles of the right pelvic limb***

- Leg muscles - 10.4 larvae/gram
- Thigh muscles – 10.02 larvae/gram
- Buttock muscles - 10.72 larvae/gram

- ***Muscles of the left pelvic limb***

- Leg muscles - 13.47 larvae/gram
- Thigh muscles - 7.92 larvae/gram
- Buttock muscles - 9.96 larvae/gram

Larvae of *Trichinella britovi* in golden jackal (*Canis aureus*) from Romania, Serbia, Iran, and Switzerland have been identified by direct trichinelloscopy and artificial digestion (4, 6, 11, 12, 16, 19).

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

The investigation of a number of 1364 wild boars from 14 hunting grounds of Vâlcea County (Romania), in the period 2017-2019, revealed the presence of the larvae *Trichinella spp*. obtained by artificial digestion in three (0.21%) of the total number of monitored animals (17).

Blaga R. et al. (3) carried out a study to identify the species of *Trichinella* which are found in wild and domestic animals in Romania.*T. spiralis* was the predominant species in domestic animals, while *T. britovi* was more prevalent in wild animals. No mixed infestations were found. The highest prevalence of infection with *Trichinella* has been detected in wolves, European wild cats, and red foxes. The geographical distribution of *Trichinella spp.* in Romania shows no specificity, with both species being present in all the studied counties (3).

The pig samples from 483 farms in Brazilian states have been examined by artificial digestion and the results revealed the absence of the species *Trichinella spiralis* which supports the statement that, in the period 2010-2013, the south of Brazil was free of *Trichinella spiralis* (20).

The results of the present study reveal that the highest larval burden is in the tongue (37.80 larvae/gram) followed by the diaphragm (21.30 larvae/gram) and the arm muscles, respectively the leg muscles (14.78 larvae/gram; 13.47 larvae/gram). The insignificant differences number of larvae has been identified in the intercostal muscles (11.20 larvae/gram) and masseters (10.80 larvae/gram).

Surprising is the number of larvae identified in the muscles of the thoracic limb, with insignificant differences between the right limb (33.49 larvae/gram) and the left one (31.57 larvae/gram). The same insignificant difference in larval load was identified between the muscles of the right limb (31.32 larvae/gram) and the left pelvic limb (31.35 larvae/gram).

Only 8.7 larvae/gram have been quantified in the neck muscles, 8.32 in the great dorsal muscles, and 9.89 in the thoracic trapezius, while the temporal muscles showed the lowest larval load (3.25 larvae/gram).

Similarly results with the present study have been highlighted by Marin et al. 2021 in the studies on the predilection sites of *Trichinella spp*. muscle larvae in red foxes (*Vulpes vulpes*). The highest number of larvae per gram tissue was found in the muscles of the tongue, arm muscles, and diaphragm (15).

Iacob O. et al provided data about the prevalence of *Trichinella spp.* infections in pigs (0.096%), horses (0.021%), wild boars (1.46%), and bears (36.76%) in the northeastern part of Romania. The dominant species was *Trichinella spiralis*, in the entire northeastern part of Romania, being identified in pigs, horses, wild boars, and bears, followed by *T. britovi* distributed in the five mountain counties, being identified only in wild boars and bears (14).

The prevalence of *Trichinella* infestation in wolves (*Canis lupus*) from Croatia has been investigated. *Trichinella britovi* was the predominant species and the larval burdens in infected animals ranged from 0.3 to 45.9 larvae per gram (2).

Conclusions

The identification of *Trichinella* larvae in a percentage of 78.57 (33/42) out of the total number of jackals examined for three years warns of the role that these wild carnivores can play in the epidemiology of this important zoonosis.

The muscles of the tongue, diaphragm, and intercostal muscles remain the muscles of choice for performing trichinelloscopy and artificial digestion.

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CANINE PARVOVIRUS - EVALUATION OF ANTIBODY TITER AND VIRAL ANTIGENIC TITER BY QUANTITATIVE IMMUNOFLUORESCENCE ASSAY

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Summary

Canine parvoviral enteritis is a severe disease and is one of the most common causes of morbidity and mortality in young dogs worldwide. Even though the disease has been known for a long time, and recently modern and diverse methods of treatment and specific prophylaxis have appeared, aimed at reducing the prevalence of the disease, the virus continues to be widely present in nature, and the morbidity and mortality of animals infected with CPV-2 remain elevated. The aim of the research was to evaluate by quantitative immunofluorescence, in canine parvovirus, the titer of antiparvovirus antibodies (post-vaccinal or post-infectious), as well as the titer of parvoviral antigens in the feces of puppies with suspected disease. To carry out the present research, samples collected from antiparvoviral vaccinated dogs, from non-parvoviral vaccinated dogs, and also from dogs with clinical signs of parvoviruses were used. Samples collected from dogs were processed by quantitative immunofluorescence to detect antibody titer and viral antigen titer. The kit Dawnsail is of real use to practitioners, considering the speed of obtaining the response, its quantitative assessment, as well as the fact that it provides a real insight into the degree of antiparvoviral protection and the viral titer in the feces.

Keywords: canine parvovirosis, immunofluorescence assay, antibody, parvovirus.

Canine parvoviral enteritis is a severe disease and is one of the most common causes of morbidity and mortality in young dogs worldwide (5, 9, 14).

The role of canine parvovirus in the pathogenesis of canine viral diarrhea is known as a disease that is considered a common health problem in dogs causing the concern of most veterinarians and dog owners throughout Romania (8, 19, 20, 25, 26).

The epidemiology of canine parvovirus is a complex and variable one, both in Romania and globally, being in a continuous dynamic, with certain geographical peculiarities, influenced by factors related to the host and, respectively, the etiological agent and not least the environment where the dogs live and where these factors may interact (4, 6, 10, 11, 13, 26). These aspects can give a particular characteristic to the evolution of the disease and its clinical manifestations (1, 3, 12, 16).

Although the disease has been known for a long time, and recently modern and diverse methods of treatment and specific prophylaxis have appeared, aimed at

reducing the prevalence of the disease, the virus continues to be widely present in nature, and the morbidity and mortality of animals infected with CPV-2 remain elevated (2, 7, 13, 17, 18).

This fact is due to the use of vaccines whose composition does not take into account the dominant CPV strain, given the limited knowledge of the distribution of new strains in a certain geographical area as well as the numerical increase of the canine population (1, 2, 4, 15, 21, 23, 24).

The lack of medical education of the owners regarding the prevention of the disease through vaccination also contributes to this situation (14, 20, 25, 26). All of the above can cause the spread of different antigenic variants of CPV-2, increasing the prevalence of the disease in a given territory at a given time (22, 27).

The aim of the research was to evaluate by quantitative immunofluorescence, in canine parvovirus, the titer of antiparvovirus antibodies (post-vaccinal or post-infectious), as well as the titer of parvoviral antigens in the feces of puppies with suspected disease.

Materials and methods

To carry out the present research, samples collected from antiparvoviral vaccinated dogs, from non-parvoviral vaccinated dogs, and also from dogs with clinical signs of parvovirosis were used.

Samples collected from dogs were processed by quantitative immunofluorescence to detect antibody titer and viral antigen titer.

Using this method, a number of 26 dog serum samples were investigated, of which 10 dogs were immunized against parvovirus and 16 adult dogs, had no known history of vaccination or parvovirus.

For the investigations regarding the presence of parvovirus in the feces of puppies with hemorrhagic diarrhea, 10 samples of feces collected from dogs brought in for a consultation in the University Veterinary Clinics in Timisoara were investigated.

For the quantification of post-vaccination antibodies, the "Dawnsail fluorescent quantitative Canine Parvovirus Antibody (CPV Ab) test kit" was also used, produced by Dawnsail Biotech, from Jiaxing City, Zhejiang Province, China (28, 29).

The results of reading the analyzed sample, for antibody titration, results consisting of the antibody titer interpretation curve, expressed in numerical values, which it converts into units, and subsequently the units are classified into one of the following categories (29): - reduced immunity, marked with "-", values between 0-2.5; - basic immunity, marked with "+", values between 2.5-10; - average immunity, marked with "++", values between 10-50; - high immunity, marked with "+++", values ≥50.

For the quantification of antigens in feces the samples were tested with the immunochromatographic test (31) and with the"Dawnsail fluorescent quantitative

Canine ParvoVirus (CPV) test kit„ also used, produced by Dawnsail Biotech, from Jiaxing City, Zhejiang Province, China (28, 30).

Reading the results with the canine parvovirus test kit allows the results to be classified into the following categories (30): - Negative (-)– between 0 and 100; - Carrier (±) – between 100 and 3000; - Weak positive (+) – between 3000 and 10000; - Medium positive (++) – between 10000 and 50000; - Strongly positive (+++) – ≥50000.

The samples were analyzed using the "Dawnsail fluorescent reader" device, produced by Dawnsail Biotech, from Jiaxing city, Zhejiang province, China. The device is equipped with WIFI and a barcode reader for the automatic reading of samples and their storage in the device's memory or the "cloud" (28).

Results and discussions

Following the investigations carried out by quantitative immunofluorescence, with the lateral flow, read with the Dawnsail device, the following results were obtained.

1. *Results obtained by quantitative immunofluorescence for the detection of antiparvovirus antibodies*

From the 26 serum samples taken from adult dogs that were vaccinated against parvovirus annually (collected at least 6 months after the last antiparvovirus vaccination), as well as from dogs with no known history of antiparvovirus vaccination and of parvovirus: 25 samples post positive (the antibody titer being between 2.5 and ≥50 units) and 1 sample was negative (values lower than 2.5 units) (29).

Samples with values higher than 2.5 units are classified into three categories of positivity (according to the kit leaflet of the manufacturer): marked with a + positive samples, but with titers up to 10 units, with ++ samples with 10 up to 50 units and with +++ samples with 50 or more than 50 units (29).

Out of the 26 samples, 10 samples came from dogs that were vaccinated previously (at least 6 months before the sample collection), so with a known immunological situation. The other 16 samples were from adult dogs with unknown immunological status. Among these 16 samples, positive results were obtained for 15 samples, and a negative response was recorded for one sample, which indicates that the kit has a good ability to detect antiparvovirus antibodies.

Within the positive category samples, the results show that all the dogs taken in the study have antibody titers that denote a basic, considered protective, antiparvoviral immunity. This immunity is the result, over time, either of antiparvoviral vaccinations (10 samples) or contact with viral strains with reduced pathogenicity, but which generated immunological restructuring resulting in the presence of antibodies in the blood samples of the dogs included in the present research.

The quantitative immunofluorescence detection of canine antiparvovirus

antibodies kit is a useful tool for practitioners in order to assess the antibody titer, as well as for establishing the moment of initiation of the post-vaccination immune response. In order to establish an optimal vaccination schedule, we consider it necessary to determine the level of antibodies before establishing the canine antiparvovirus immunization protocol.

2. *Results obtained by quantitative immunofluorescence for the detection of parvoviral antigen in feces*

The results obtained with the kit used for the quantitative titration of the parvoviral antigen showed that all 10 fecal samples, taken from dogs with clinical signs of parvovirosis, gave a positive response for the parvoviral antigen, both by immunochromatographic test and also by quantitative immunofluorescence.

The 10 fecal samples taken from dogs with clinical signs of parvovirus were classified as follows: 3 samples were classified as originating from parvovirus-carrying puppies, and the remaining 7 samples were classified as originating from positive puppies at parvovirus antigen. In turn, the positive samples were assigned as follows: 5 weakly positive samples, 1 medium, and 1 intensely positive sample each.

Unlike the results provided by the immunochromatographic test (31), in which animals can be classified into only 2 categories (positive and negative), the results obtained with the immunofluorescence kit, used for the quantitative titration of parvoviral antigen, allow a more extensive systematization of the response obtained, regarding the evaluation of the antigenic titer in the feces, because the samples are classified into one of the 5 categories: negative, carrier, weakly positive, medium positive and strongly positive (30).

Samples whose values are below the limit of 3000 units are considered to come from carrier dogs, and samples whose values exceed 3000 units are considered positive, but they differ from each other in the intensity of positivity, which can be: weak, medium or strong, marked respectively with +, ++ and +++ (30).

The results obtained with the quantitative immunofluorescence kit, used for the titration of parvoviral antigen, read with the Dawnsail device (28), thus allowing practitioners to better manage clinical cases of parvovirosis, given that the test provides, in a short period, valuable information on the viral antigenic titer in feces, which is in close correlation with the intensity of viral aggression on infected puppies.

Conclusions

The kit Dawnsail is of real use to practitioners, considering the speed of obtaining the response, its quantitative assessment, as well as the fact that it provides a real insight into the degree of antiparvoviral protection and the viral titer in the feces.

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POSTVACCINAL IMMUNE RESPONSE IN CANINE PARVOVIROSIS

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Summary

Canine parvovirosis, despite extensive vaccination, remains an important cause of mortality in dogs, especially in puppies. There is an extremely large variation in the response of organisms to antiparvoviral immunization and also there is an extremely wide variation in the response of organisms to infection. The aim of this research was to evaluate the post-vaccination immune response in puppies vaccinated with two vaccines from different companies. Following this study, it can be stated with certainty that in establishing the scheme of vaccination of puppies against parvovirus mainly, but also against the other infectious diseases that benefit from immunoprophylaxis, it must be taken into account that several factors can influence the effectiveness of vaccination. That is why vaccination can be considered a "personalized" action in which a series of individual factors are involved: age, sex, breed, the living environment of the puppy, as well as the infectious pressure in the respective geographical area. However, we cannot recommend a standard canine parvovirus vaccination schedule that covers all existing situations. To establish an optimal vaccination schedule, we consider it necessary to determine the level of maternal antibodies, before establishing the canine parvovirus immunization protocol, considering that maternal antibodies interfered with the post-vaccination immune response, this fact being obviously in puppies vaccinated with B schedule.

Keywords: parvovirus vaccine, immunization, canine parvovirosis.

Canine parvovirosis (26), despite extensive vaccination, remains an important cause of mortality in dogs, especially in puppies (1, 4, 13, 18). There is an extremely large variation in the response of organisms to antiparvoviral immunization and also there is an extremely wide variation in the response of organisms to infection (7, 10, 11, 14). Although the animals develop clinically inapparent infections in many cases, there are still situations in which the clinical expressions are extremely obvious (5, 16, 19). Morbidity and mortality are difficult to estimate (13, 18, 23), being able to vary within extremely wide limits, depending on numerous factors involved in the epidemiology of the disease (age, stress, race, co-infections, associated parasitosis, immune status, etc.). In kennels, in certain nests, morbidity and mortality can vary from 10 to 90% (17, 20, 21). The general measures of non-specific prophylaxis are extremely difficult to apply due to the degree of spread of the virus in the environment and its particular resistance (22, 24). Some authors believe that, regardless of the degree of compliance with the general prophylaxis rules applied, they often prove to be ineffective, the only effective measures to

prevent the disease being the immunoprophylactic ones (2, 6, 8).

Vaccination with vaccines containing live attenuated strains is the most widely used method of immunization of puppies against parvovirus (3, 8, 14, 15).

The numerous failures resulting from anti-parvoviral vaccinations reported in the specialized literature are based on the phenomenon of interference between the maternal antibodies and the vaccine antigen, a fact that causes an inappropriate seroconversion (6, 13, 18, 24).

In practice, numerous vaccination schemes are used, with a greater or lesser number of boosters or with vaccines produced by different companies. Very often, vaccinated puppies get sick with parvovirus, they even develop a serious clinical picture and the evolution ends with the death of the puppies (1, 9, 12, 25).

The aim of this research was to evaluate the post-vaccination immune response in puppies vaccinated with two vaccines from different companies.

Materials and methods

For the immunization of the two groups of puppies which were included in the study, the following products were used:

- a vaccine containing modified live viral strains, currently used to immunize puppies against parvovirus;
- a bivalent vaccine that contains a live virus against canine distemper (lyophilized component) and an inactivated virus against canine parvovirus (liquid component).

The first vaccination was done when puppies are between 6-17 weeks of age when they were presented to the clinic for the first vaccination; after two weeks they received the second dose of vaccine and the third dose was done after another 2 or 3 weeks

Blood was collected by venipuncture from the jugular or antebrachial vein into clot activator vacutainers to facilitate serum expression. The amount of blood collected was between 1.5 and 3 ml/puppy/collection. After that, the samples were left at room temperature (23℃) for several hours to allow serum expression. After expressing, the serum was decanted into Eppendorf tubes. The samples were kept in the freezer until the time of testing.

The INGezim PARVO CANINO 15.CPV K.1 ELISA kit, Ingenasa, Spain, was used for post-vaccination antibody titration, an indirect immune-enzymatic assay (27).

Results and discussions

The results obtained from the titration of the antibodies by the ELISA assay are presented in graphs.

Fig, 1 presents the comparative dynamics of the anti-parvovirus antibody curve at the three samplings performed, after immunization with schedule A and

schedule B.

Analyzing the data presented in Fig. 1 can see the difference between the O.D. of the samples. In the case of vaccine A, a trend of continuous increase in the antibody titer can be observed, the increase being accentuated after the administration of the second vaccine so that at the third collection the titer of the antibody was very high.

In the case of vaccine B, the dynamic is different, such that after the first administration of the vaccine, the geometric mean of the titers decreased and increased after the second vaccination, however, the geometric mean of the O.D. obtained at the third sampling was much lower than in the case of vaccine A. In our opinion, the vaccination of puppies with B-scheme provides lower anti-parvovirus protection. Anyway, further investigations with a significantly higher number of samples are needed, as it cannot be stated with certainty that B-vaccine does not induce an immune response as strong as vaccine A.

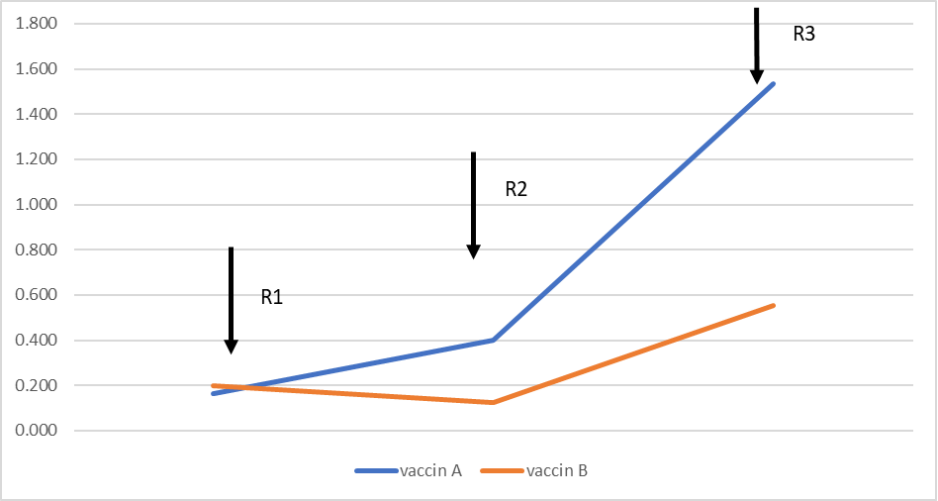

Fig. 1. Comparative dynamics of the evolution of the geometric means of O.D. to the two immunization schedules used

Further investigations regarding immune response in puppies correlated with the fact that the mothers were or were not vaccinated would be needed (17). The results presented in this study show that in puppies that had high levels of maternal antibodies at the first test, maternal antibodies interfered with the post-vaccination immune response, causing low O.D. values in the second and third samplings.

In the case of both vaccination schedules, extremely high antibody titer values were obtained, especially after the administration of the second dose of

vaccine, as well as low antibody titer values, values considered non-protective.

Following this study, it can be stated with certainty that in establishing the scheme of vaccination of puppies against parvovirus mainly, but also against the other infectious diseases that benefit from immunoprophylaxis, it must be taken into account that several factors can influence the effectiveness of vaccination (19). That is why vaccination can be considered a "personalized" action in which a series of individual factors are involved: age, sex, breed, the living environment of the puppy, as well as the infectious pressure in the respective geographical area, etc (1, 2, 10, 13, 18, 23). However, we cannot recommend a standard canine parvovirus vaccination schedule that covers all existing situations.

We also consider that is important to determine the antibody titer of the puppies before starting the vaccination protocol, to evidence the level of maternal antibodies (if any), and to estimate the optimal time of the first vaccination dose (1, 17).

Both vaccines produced antibody titers considered protective, only after the second vaccination, so 90% of puppies vaccinated with A schedule and only 55% of puppies vaccinated with B schedule can be considered protected against parvovirus infection canine, having titers with protective values of canine anti-parvovirus antibodies.

In puppies vaccinated with the B schedule (first dose consisting of inactivated vaccine), no anti-parvovirus antibodies were detected 14-21 days after the first vaccine dose, which demonstrates the poor immunogenicity of the inactivated vaccine.

Conclusions

To establish an optimal vaccination schedule, we consider it necessary to determine the level of maternal antibodies, before establishing the canine parvovirus immunization protocol, considering that maternal antibodies interfered with the post-vaccination immune response, this fact being obviously in puppies vaccinated with B schedule.

Acknowledgment

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COMPUTED TOMOGRAPHY IMAGING IN CANINE RIB NEOPLASIA: A TWO CASE STUDY

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Summary

Radiology is used as a means of diagnosing both intrathoracic, as well as systemic conditions, although the thorax is one of the most challenging areas to evaluate radiographically, due to several reasons (e.g. positioning, superimposition). Computed tomography has the advantage of not dealing with superimposition, and is paramount to surgical planning for masses located on thoracic walls (e.g. rib neoplasia) especially when radiology failed to properly determine the masses' boundaries. Osteosarcoma and chondrosarcoma are the most frequently seen tumors affecting the ribs, although primary rib neoplasia is rarely seen in dogs. The current study presents 2 cases – an 11-month-old Doberman Pinscher presented with dyspnea and anorexia, and a 10-year-old shepherd-mix female dog, presented with clinical signs suggestive of spinal cord compression; both patients also had a common clinical sign: a firm, broad-based mass, fixed to the distal end of different ribs. Native and post-contrast CT scans were conducted on both patients, whilst the Doberman Pinscher also benefited from a 4-view radiographic study. Clinical signs, as well as radiographic and CT findings for these two dogs, are presented in the present study.

Keywords: computed tomography, canine, rib, neoplasia.

Primary tumors affecting the ribs in dogs are rare, in comparison to primary bone tumors affecting the appendicular skeleton. Predilection regarding breed or sex is not known, with these tumors being identified also in young dogs (15). Large-breed dogs (e.g. Boxers) are the most commonly affected (11).

Osteosarcoma is the most common primary rib tumor, alongside chondrosarcoma (2, 3, 4, 7, 9, 13, 14, 15, 17, 18). Other types of neoplasia - fibrosarcoma, haemangiosarcoma, osteoma, multilobular tumors (2, 3, 15), as well as chondroma (14, 15) - have also been reported. Osteochondroma, another type of neoplasia affecting the ribcage, usually affects young dogs (2, 3).

Clinical signs often include the respiratory system – dyspnea (4, 7, 15) and cough, secondary to lung compression from the mass/masses, and/or hemothorax, firmly attached thoracic mass, and in some cases lameness of the front limbs (15). A late diagnosis is usually established due to a more pronounced intrathoracic expansion, rather than an external one (15, 17).

Primary rib tumors affect the distal third of the rib/near the costochondral junction (15).

In patients with pleural effusion of varying degrees, thoracic masses may be

masked by the fluid (6, 17). In some cases, evacuation of the pleural fluid may be needed, followed by repeat radiographs (17).

Thoracic wall masses invading the thoracic cavity may present with an extrapleural sign (5, 12, 16, 17). This specific sign appears as an intrathoracic mass with a convex margin facing the lungs, with cranial and caudal margins tapering along the thoracic wall. In order to properly identify an extrapleural sign, the x-ray beam must strike the intrathoracic part of the masses tangentially (17). If the standard thoracic views (dorsoventral, ventrodorsal, and lateral views) offer no clear insight into an extrapleural sign, oblique views should be obtained (17).

Materials and methods

The study was conducted in the Computed Tomography Laboratory of the Faculty of Veterinary Medicine from Timişoara.

Two canine patients underwent CT examination of their thoracic cavities, and vertebral column respectively. The patients were an 11-month-old Doberman Pinscher and a 10-year-old shepherd-mix female dog. The former patient, also benefited from a 4-view radiographic study, before CT scanning.

The Doberman Pinscher was referred for thoracic radiographs after being consulted in the Internal Medicine Clinic of our Faculty. the 4-view radiographic study consisted in performing 2 lateral radiographs (in right and left recumbency), a ventrodorsal radiograph, and a dorsoventral one, respectively. After the radiographic study was concluded, discussions underwent with the referring physician and owners regarding the benefit of a CT scan.

The Shepherd-mix dog was referred to our service with suspicion of the intervertebral disc disease and secondary spinal cord compression –according to clinical and neurological signs - by a private practice veterinary doctor, and concerns regarding a broad-based thoracic wall mass. The same patient had a history of mammary gland tumors on the left mammary row, which was completely surgically removed by the same veterinary doctor. Tissue samples of the tumors were sent for histopathology testing following surgery.

For the radiographic study, a Siemens Multix Swing was used, with the Doberman patient non-sedated (Fig. 1).

Anesthesia was performed using a combination of medetomidine (20-40 μg/kg) and propofol (0.5 – 1.5 mg/kg). Recovery was obtained with atipamezole (at 5 times the dose of medetomidine).

Both native and contrast studies were conducted and images were obtained using multiplanar reconstruction (MPR) at 0.6 mm slice thickness, and 3D volume rendering technique (VRT). Ultravist 370 (Iopromide, Bayer), at a dose of 1 ml/kg, was the contrast medium of choice used for the contrast-enhanced CT scans.

Fig. 1. Siemens Multix Swing

A Siemens Somatom Definition AS 64 slice CT scanner was used to scan the patients, under general anesthesia (Fig. 2).

Fig. 2. Siemens Somatom Definition AS 64

Results and discussions

Recovery from anesthesia was uneventful in both patients.

The Doberman Pinscher was dyspneic, and anorexic, and had a non-painful, firmly attached chest wall mass. The 4-view radiographic study revealed severe pleural effusion and secondary lung collapse, pulmonary alveolar pattern in the cranio-ventral compartment, and an irregularly shaped distal 3rd of the left 9th

rib, showing signs of irregular periosteal reaction and osteolysis (Fig. 3). Radiographic aspects suggestive for a thoracic wall mass consisted in the presence of a soft tissue swelling in the area of the left 8th, 9th and 10th ribs, slightly extending outwards, and the presence of an extrapleural sign centered on the 9th rib.

Fig. 3. Dorso-ventral thoracic radiographic view of the Doberman Pinscher.
There is evidence of lung collapse due to pleural effusion, irregular periosteal reaction, and osteolysis of the distal third of the left 9th rib

CT scanning was performed, and revealed bilateral severe pleural effusion, severe lung collapse, and a large broad-based soft tissue mass originating in the left 9th rib, with an intrathoracic part that was greater in size than its exterior part (Fig. 4). Postcontrast study revealed mild heterogenous contrast enhancement of the mass, but aided nonetheless in delimitating the margins of the tumor.

The soft tissue mass associated with the left 9th rib was not easily seen on radiographs, due to the superimposition of the pleural effusion with the mass itself (Fig. 4).

Fig. 4. Transverse view of the thoracic cavity at the level of the 9th ribs.
A large soft tissue mass with mild contrast enhancement. The margins of the tumor are somewhat clear, due to silhouetting with the pleural effusion (white thin arrows). Severe lung collapse and a large amount of pleural effusion. Osteolysis and irregular periosteal of the distal 3rd of the left 9th rib are also seen

The second case, a 10-year-old female intact Shepherd-mix dog, was referred to us for the suspicion of the intervertebral disc disease and secondary spinal cord compression in the lumbar area. The patient was paraplegic, with proprioceptive deficits on the hindlimbs, weak reflexes bilaterally of the hindlimbs, and the presence of the panniculus reflex all the way to the mid-lumbar segment. Concerns regarding a non-painful, broad-based mass on its thoracic wall were also discussed, so the thoracic cavity was included in the CT scan.

Upon examining the vertebral column and spinal cord, a severe periosteal reaction was identified at the level of the 3rd lumbar vertebra, centered on its body. A soft tissue component was also noted, part of which was protruding inside the vertebral canal and causing severe spinal cord compression (Fig. 5). Mild osteolysis was also noted, especially of the dorsal cortex of the vertebral body.

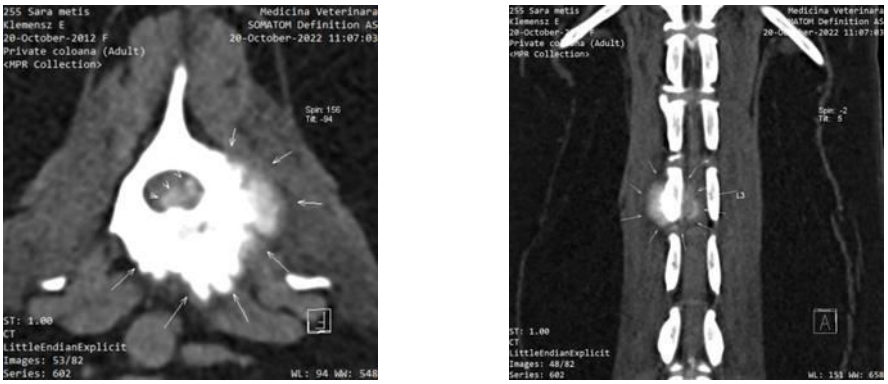

Fig. 5. Contrast-enhanced transverse (left) and dorsal (right) MPR views centered on the 3rd lumbar vertebra, showing severe periosteal reaction extending ventrally, laterally, and invading the vertebral canal as well

Thoracic examination revealed a large, mixed-aspect mass arising near the costochondral junction of the left 4th rib, with both an outward expansion and an intrathoracic component, as well. Pleural effusion was not noted despite the large size of the tumor (Fig. 6).

Fig. 6. Contrast-enhanced transverse (a) and dorsal (b) MPR views centered on the left 4th rib revealing the large mass originating near the costochondral junction, expanding both outwards, as well as intrathoracic

VRT helps create a three-dimensional view of the patient's area of interest – the future surgical field – namely, of the tumor size, expansion, and boundaries, as well as any healthy organs/tissues that may be secondary involved and/or affected (Fig. 7). Intercostal spaces may appear wider than normal (11).

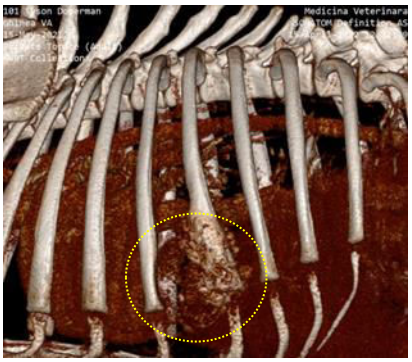

Fig. 7. Lateral view of the 3D reconstruction of the Doberman revealing a widened intercostal space between the 8th and 9th ribs, as well as a concave deformity of the caudal aspect of the 8th rib due to chronic compression by the tumor

3D reconstruction is also helpful in understanding the severity of the lung collapse secondary to pleural effusion; for example, an air/gas filter helps identify air/gas-filled structures, in contrast to solid structures (Fig. 8).

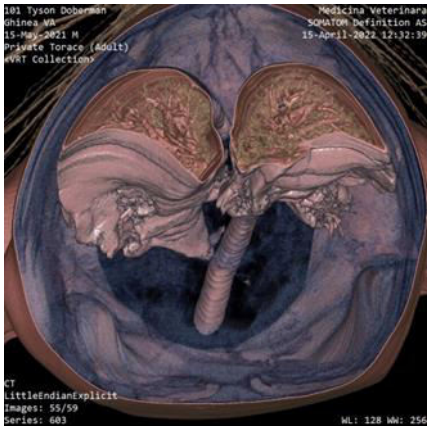

Fig. 8. Caudal view of the 3D reconstruction using an air/gas filter of the thoracic cavity of the Doberman Pinscher, revealing the air-filled structures. Note dorsally and bilaterally the severely collapsed air-filled lungs

In the second patient, several other predominantly productive lesions were observed on other left-sided ribs (proximal part of the ribs), as well as another lesion located on the proximal part of the left scapula; the sternal lymph node was also

enlarged in this case (Fig. 9).

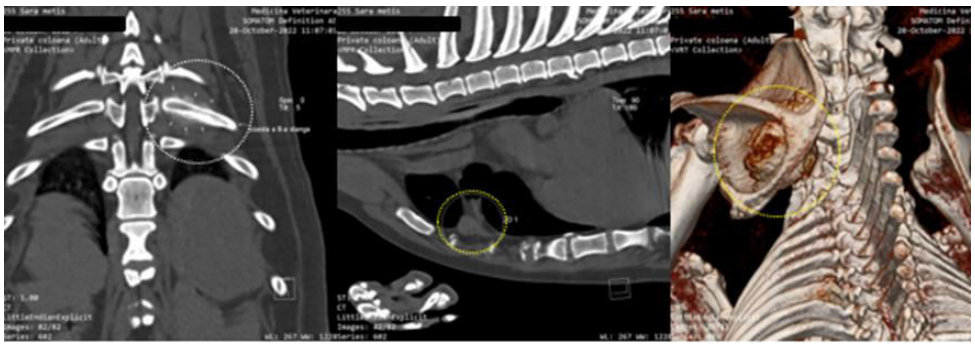

Fig. 9. Several other lesions located on the left 8th rib, sternal lymph node, and left scapula

Secondary rib neoplasia is usually smaller in size and arises on the proximal/middle part of the affected ribs (15).

Metastatic rib neoplasia may also appear in its early stages as fractures that have healed (11).

Benign tumors originating in the ribs are rare (e.g. osteoma, chondroma) and do not have an aggressive aspect, often leading to changes in the local anatomy, and typically do not cause osteolysis (18).

Both masses were large, located near the costochondral junction, and had a mixed aspect – both destructive and productive – all of which are suggestive of primary malignant rib tumor (15, 18).

Even though primary rib neoplasia may give rise to lung metastases (15), no such lesions were seen in any of the patients.

In the case of the Shepherd-mix dog, the smaller rib lesions, those located on the left scapula and the vertebral one were considered to be secondary metastasis originating from the large rib mass. The patient had several mammary tumors, but following a histological diagnosis of presumed mixed adenoma, it was considered that these lesions were not secondary to the mammary tumors.

CT examination of the thoracic cavity, following contrast medium administration, is likely more valuable in staging and planning for surgery (5).

Before surgical excision, it is mandatory to obtain a histological diagnosis, since the prognosis is dependent upon the histologic type (1).

Resection with wide margins is needed to lower the risk of incomplete resection, but in dogs with primary osteosarcoma adjunctive chemotherapy is recommended (9).

Even under such requirements, further diagnostics were not pursued in any of the patients presented here, and euthanasia was performed shortly after the

diagnosis, given the patients' poor general condition.

A late diagnosis is usually established in such patients (15, 17). In one study, less than 10% of the canine patients diagnosed with primary osteosarcoma had lived more than 4 months following the diagnosis (4).

A nearly similar period of 17 weeks of survival after surgery was reported in dogs with osteosarcoma (1).

Surgical resection alone for primary rib chondrosarcoma has a very good prognosis, but the prognosis is guarded in the case of primary rib osteosarcoma (5, 9).

Dogs with primary rib chondrosarcoma have a higher median survival time, as compared to dogs with other types of primary rib neoplasia (9, 10).

Regarding laboratory findings, increased levels of serum alkaline phosphatase in dogs with osteosarcoma affecting flat bone – ribs included – are associated with decreased survival times (8).

Conclusions

CT is recommended in patients with masses firmly attached to the thoracic walls and is superior to radiological studies.

CT is useful in differentiating tumor boundaries from healthy soft tissues, and pleural effusion, and is crucial for surgical planning.

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EFFECTIVENESS OF RABIES IMMUNOPROPHYLAXIS IN WILDLIFE BY APPLYING VACCINES FROM AIRPLANE

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Summary

The study aimed to analyze the epidemiological situation regarding rabies in the Republic of Moldova from 2010 to 2022. The obtained results showed that in the Republic of Moldova rabies evolves endemic, and the annual number of rabies cases in animals has dragging variations with value reaching incidences from 58 cases (2011) to 167 cases (2015), is characterized by successive waves of increasing the number of sick animals with a periodicity of 2 to 3 years. The presented data also showed a correlation between the increase in the number of rabies cases in domestic animals in accordance with the number of cases of rabies in wildlife (in foxes). The highest share of rabies in animals was recorded in foxes; these animals are considered the main factor in the spread of the disease. At the same time, the wildlife vaccination program (2020-2022) through the administration of baits from the plane, has contributed to reducing the number of rabies cases considerably in wildlife and domestically animals and the immunological efficiency of the vaccinated foxes was the presence of antibody titers in more than 50% of the shouted exanimated foxes.

Keywords: rabies**,** Moldova**,** fox, Lisvulpen vaccine**.**

Rabies is a fatal but preventable viral disease. It can spread to people and pets if they are bitten or scratched by a rabid animal. Around the world, rabies is mostly found in wild animals like bats, raccoons, skunks, and foxes. However, in many other countries dogs still carry rabies, and most rabies deaths in people around the world are caused by dog bites (4, 12, 19, 20).

Rabies is a zoonosis of virotic origin that has the highest lethality rate in both animals and humans infected with the rabies virus. Notifications of the incidence of the disease annually come from more than 150 countries around the world, and the number of deaths due to rabies is over 50,000 people annually. At the same time, the number of people vaccinated due to bites caused by sick or suspected animals infected with the rabies virus exceeds 15 million persons every year. The rabies virus particularly affects the central nervous system (brain and spinal cord). It occurs rarely in vaccinated pets but can be easily contracted if an unvaccinated pet comes in contact with another sick or wild animal and is bitten or scratched by it. Humans can be contaminated by bites, produced by both contaminated domestic and wild animals (2, 4, 5, 6, 7, 14, 16).

Consequently, foxes enter in localities and in animal shelters during the day and allow humans to approach them. Moreover, without becoming aggressive they

bite animals and humans and have a fixed gaze sometimes with an insecure gait followed by paralysis and death.

Another important source of the disease as well as a potential reservoir of the virus represents the stray dogs in urban areas that bite humans and various species of animals. However, the measures taken to reduce their number and expose them to prophylactic vaccinations are evolving slowly and require additional financing sources (1, 11, 15).

Given the important social and economic impact of rabies, the disease is constantly monitored by the veterinary service and is being included in the plan of strategic veterinary sanitary measures in the country which provides concrete measures regarding surveillance, prevention, and control of the disease in susceptible animals and assures the population protection from contamination (3, 13).

Materials and methods

The research was conducted in the period starting from 2010 to 2022 during which extensive epidemiological data studies and analysis were performed on the incidence of rabies in domestic animals and wildlife in the Republic of Moldova. Clinical cases of rabies in domestic animals with rabies were examined, as well as cases of rabies in wild animals were recorded and investigated. Nonetheless, in case of suspicions of rabies, to confirm the diagnosis, samples of pathological material (animal head) were sent to the national reference laboratory Public Institution Republican Center for Veterinary Diagnosis.

The confirmation of the diagnosis was made by direct immunofluorescence examination stained with specific fluorescent conjugate (Lyophilized, adsorbed anti-rabies nucleocapsid conjugate) for testing for *Lyssavirus Genotype 3* (*Mokola virus*).

Results and discussions

The Republic of Moldova is one of the countries in the European countries region which has a comparatively high number of rabies cases. From 1952 to the present, rabies remains an endemic zoonosis in the territory of the Republic of Moldova with a different incidence of disease; having a higher frequency in wild animals, mainly represented by the red fox.

In the Republic of Moldova (Fig. 1), rabies evolves endemic, and the annual number of rabies cases in animals has variations with incidences from 58 cases (2011) to 167 cases (2015), is characterized by successive waves of increasing the number of sick animals with a periodicity of 2 to 3 years.

In 2020 was launched the bilateral program between the Republic of Moldova-Romania, aimed to vaccinate wildlife animals against rabies by the combined method, manually and by plane.

The vaccination area should be as large as possible and preferably should

include the entire rabies-affected (endemic) area. Oral rabies vaccination of foxes and/or raccoon dogs should be carried out twice a year, in spring and in autumn. Spring and autumn vaccination campaigns should be preferably conducted from the end of March to the beginning of June and from late September until the end of October.

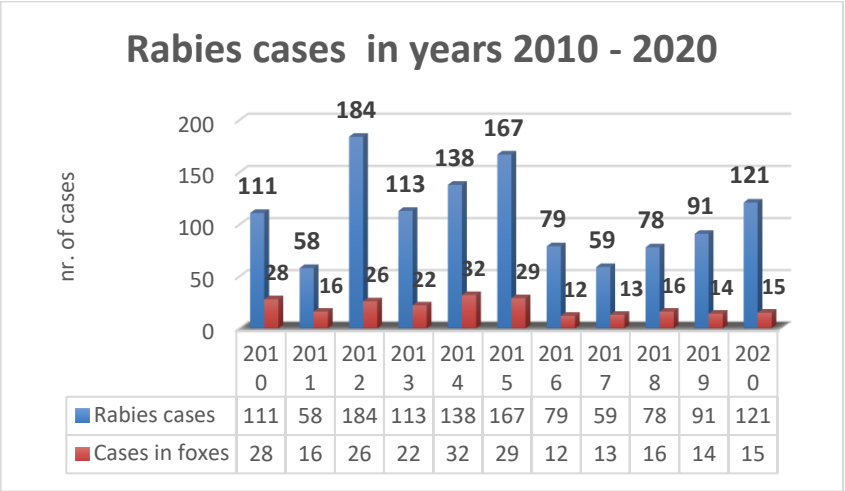

Fig. 1. Rabies cases in the Republic of Moldova during 2010- 2020

Aerial distribution of baits using fixed-winged aircraft or helicopters is the method of choice.

The classical flight pattern consists of parallel flight lines set approximately 500 meters apart.

The distribution pattern of baits should take into account habitat and landscape features. Under certain circumstances, e.g. extreme topographical features (fragmented landscapes) or persistent residual foci, deviation from parallel flight line patterns might be acceptable provided that the requested bait density per km² is guaranteed.

The use of GPS systems, computer-supported recording of flight routes, and coordinates of bait droppings during aerial distribution is necessary for quality control and detailed analysis of data. For the manual distribution of baits, an equivalent recording of bait placements should be used.

Furthermore, GIS technology should be made available in order to transform the data recorded during bait distribution into bait density maps. These maps allow for a proper evaluation of the bait distribution required and enable to establish the appropriateness of bait coverage both in terms of territory and density. Accordingly, additional flights could be required in order to guarantee appropriate bait coverage

in some areas.

Based on experience, the minimum bait density to be applied should not be less than 20 baits/km². In case of high population densities, setbacks, and persisting residual foci, an increased bait density of up to 25-30 baits/km² in combination with reduced flight line distance or adapted flight line patterns should be considered as a corrective action.

For the years 2020-2022 were used Lisvulpen vaccine produced by the Bioveta company Czech Republic (18).

The vaccine was distributed by the small plane type CESNA.

The data presented in Table 1 reports that during the years 2020-2022, 2 wildlife vaccination campaigns (foxes) were carried out, and the vaccination baits were distributed by plane in the forest files and partially by hand, the baits being placed at the foxes' dens.

In total, during this period, 156,600 doses were distributed by the manual method and 2040,832 by plane.

Table 1

Vaccines distributed in the years 2020 – 2022

Year	Small plane distribution	Manual distribution
2020 I campaign	537858	52200
2020 II campaign	574443	52200
2021 I campaign	0	0
2021 II campaign	611555	52200
2022 I campaign	316976	0
2022 II campaign	450600	0
Total	**2040832**	**156600**

Fig. 2 shows the map of the Republic of Moldova with the marking of the flight lines of the plane for the distribution of vaccine baits. It was taken into account that the baits should not be distributed over lakes or urban and rural settlements.

Monitoring the effectiveness of ORV is based on three main pillars:

- determination of bait uptake by detecting the presence of biomarkers,
- determination of seroconversion by detecting rabies-specific antibodies in target animals (foxes and raccoon dogs) sampled in vaccination areas, and
- enhanced rabies surveillance in all animal species (wild and domestic): decrease in rabies incidence is the key index for the success of an ORV program.

For investigations in the first two pillars, sampling should focus on animals from the hunting bag; here, it is considered sufficient to test 4 animals per 100 km2 annually from vaccinated areas. Sampling should be conducted homogeneously to avoid bias in the results to be obtained. Whenever possible blood/body fluid and

teeth/bone tissue samples for serological testing and detection of biomarkers, respectively, should be taken from the same animal.

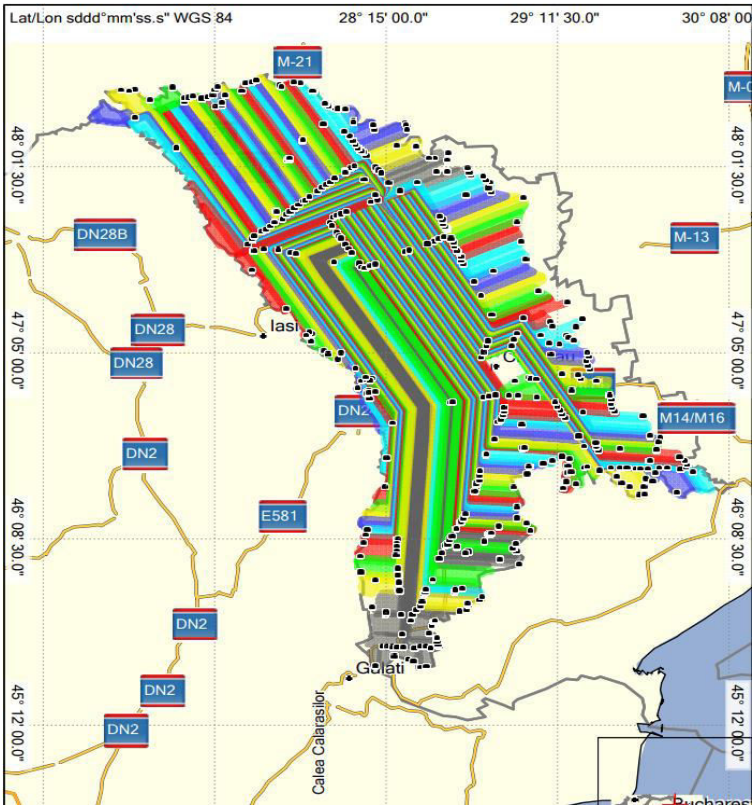

Fig. 2. The plane distribution (flight lines)

To assess the immune response due to vaccination, sero-neutralisation tests such as RFFIT (Rapid fluorescent focus inhibition test) and FAVN (Fluorescent antibody virus neutralization test) test should be used. However, those tests are sensitive to cytotoxicity, virucidal effects, and bacterial contamination, particularly with poor-quality samples. Alternative serological assays are commercial direct or indirect rabies ELISA test kits validated for field samples of wild animal origin. When using those tests attention should be paid in terms of sensitivity and specificity. The EURL for rabies serology should be contacted for further information and for possible interest in inter-laboratory serological testing.

For proper epidemiological evaluation of ORV, stratification of data should be considered. Therefore, basic data such as the date of sampling, geo-location of

hunted animals, age, and results of laboratory investigations should be collected.

Table 2

The effectiveness of vaccination in wildlife condition (2020)

Vaccination campaigns	No. of mandibles	No. thoracic liquid	Tetracycline positive	% positive animals	Antibodies positive	% positive animals
I (2020)	95	82	80	-	13	**-**
II (2020)	199	160	180	-	97	**-**
Total (2020)	**294**	**242**	**260**	**48.5**	**110**	**20.56**

Analyzing the data in Table 2, it can be mentioned that after vaccination, in 2020, were taken for examination 294 mandibles from shouted foxes and 242 thoracic fluid samples. As a result of the research, it was established that the presence of tetracycline in the mandibles was established in 260 samples, which constituted 48.5% of the examined samples, and specific antibody titers were established in 110 samples, which constituted 20.56%.

Table 3

The effectiveness of vaccination in wildlife condition (2021)

Vaccination campaign	No. of mandibles	No. thoracic liquid	Tetracycline positive	% positive animals	Antibodies positive	% positive animals
I (2021)	-	-	-	-	-	-
II (2021)	218	162	187	-	52	-
Total (2021)	**218**	**162**	**187**	**74.31**	**52**	**27.8**

In 2021 (Table 3), were taken for examination 218 mandibles from shouted foxes and 162 thoracic fluid samples. As a result of the research, it was established that the presence of tetracycline in the mandibles was established in 162 samples,

which constituted 74.31% of the examined samples, and specific antibody titers were established in 52 samples, which constituted 27.8%.

Some authors mention that, in the population of wild animals in Romania, most cases of rabies were recorded in foxes, where annually at least 66.66% of the cases of rabies from wild animals were provided by foxes (8, 9, 10, 17).

Regarding the situation in Europe, from the primary data analyzed, it can be observed that even here the cases of rabies in wild animals predominate compared to domestic ones, compared to the total number of cases (19, 21).

Considering the increase in the percentage of cases of rabies in foxes, in the last period and considering the high percentage seen as a whole throughout the study period, as well as the impact of the number of cases of rabies in foxes on the number of cases from domestic animals, the initiation of the national program for rabies immunoprophylaxis in foxes was justified, in accordance with the programs already applied in other countries of the European Union.

Conclusions

The most frequent cases of the disease were registered in foxes, which are also considered the main vector of disease spread; having variations of confirmed cases that ranged from 14 to 32 based on laboratory investigations, which constituted from 15.4% to 23.2% of the total number of confirmed rabies cases annually.

The distribution of vaccines by plane led to a significant reduction in the number of rabies cases in wildlife (foxes), which is reflected in the decrees of rabies cases in domestic animals and to create of an immune background with antibody titers within the limits of up to 27.8% in the animals subjected to serological investigations.

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THE ROLES OF THE DIGESTIVE MICROBIOTA IN PETS

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Summary

The microbiota is composed of over 500 species of bacteria, viruses, fungi, and protozoa. The last few years have shown that there is a great interest in studying the microbiota among veterinarians as well. In this article we'll review a series of recent research and discoveries regarding the digestive microbiota and its roles in the various functions of the body such as metabolism, protection against pathogens, education of the immune response, synthesis, and or facilitation of the synthesis of key nutrients in the maintenance of physical health, in the development of obesity, in dermatology, the microbiome is a "metabolic organ" with an essential role in maintaining health and preventing or treating diseases in humans but also in pets. By maintaining the health of the body's basic functions, the microbiome can directly or indirectly influence most of the host's physiological functions and multiple organs.
Keywords: microbiome, dysbiosis, digestive health, fiber.

The digestive microbiota is the population of microorganisms present at the digestive level. The microbiome is the interacting system made up of the genome of the host cells and the resistant microbes present is the current name of the gut microbiome. Often these two terms have been used in parallel or interchangeably to express the same thing, but recently there is this clear distinction between the terms.

The digestive tract of mammals, in addition to bacteria, which constitute over 98% of the total microbiota, also contains other species of micro-organisms such as fungi, protozoa, viruses, bacteria, and archaea. Molecular studies have provided in-depth data on the diversity of these microorganisms including among healthy animals. Studies also highlight the interactions between microbes and their influence on the host, but their role in gastrointestinal diseases remains unclear for now (4.)

Subgroups of the microbiome include the virome (viruses), the mycobiome (fungi), and the archaeome (archaea; bacteria-like organisms, but represent a separate, distinct domain). The microbiome is a dynamic environment that has complex and interconnected interactions with metabolism (4).

The virome includes viruses that infect bacteria (bacteriophages) within the microbiome as well as host cells. Bacteriophages make up the majority of the virome (3, 18).

At this time, research on the virome is very limited, but research on changes in the fecal virome has been identified in dogs with chronic enteropathies or those suffering from acute diarrhea. Likewise, research on the mycobiome is extremely limited at the moment (3).

The genes of the microbiome encode proteins for the survival of the host but which are not encoded by the mammalian genome, thus the microbiome is considered the "forgotten organ" or the "hidden organ" (11).

Healthy gut microbiota

The healthy gut microbiota can be described as the complex of bacteria that colonizes a defined area of the digestive tract in humans or animals and has not been affected by disease or any drug intervention Like any other organ, the microbiome has a physiology and a pathology, and health individual (and collective) might be affected when the composition of its population is unbalanced. The diagnosis of microbiome-driven conditions involves metagenomic studies. The therapeutics of pathologies induced by the microbiome or rather those induced by microbiome disorders, also called dysbiosis, include microbiota or bacterial transplantation, a recently available technique that is continuously growing. Recent molecular studies, based on comparative genetic analyzes on 16S rRNA, have revealed that the gastrointestinal tract of mammals harbors from several hundred to several thousand bacterial genera and types. It is estimated that the gut of mammals contains about 10^{10} to 10^{14} microorganisms, almost 10 times more than the total number of cells that make up the body of their host (2, 3, 9, 18).

These digestive bacteria have an important role in defending the health of the host, acting as a protective barrier against invading pathogens, helping digestive processes, providing energy from food, providing nutritional support for enterocytes, and stimulating the development of the immune system. Molecular studies have helped to improve our understanding of the composition, dynamics, and functioning of the intestinal ecosystem in cats and dogs (2, 3, 9, 18).

Pets harvest at the digestive level 5 major bacterial genera and the most represented fila we may find in Table 1 (3, 18).

The microbiota or the dysbiotic digestive microbiome has been associated with several conditions such as gastrointestinal issues, obesity, diabetes, metabolic syndrome, cardiovascular diseases, immunological disorders, liver diseases, but also behavioral changes (3, 18).

The roles of the microbiota

1. Role for intestinal development

As we specified previously, the intestinal microbiota plays an important first role in intestinal development. The pathogens present could negatively change intestinal morphology. It has also been demonstrated that SCFAs (Short chain fatty acids) such as butyrate, propionate, and acetate, are products of the intestinal microbiota that contribute to the growth and proliferation of enterocytes. The intestinal microbiota influences intestinal morphology both in humans and in all species of mammals as well as in birds. Butyrate, or butyric acid, is the primary energy source for the colonic epithelium and has also been shown to play a critical

role in maintaining colonocyte homeostasis and the morphological development of intestinal villi (3, 17).

Table 1

Bacterial families and the most abundant representatives in the digestive microbiota -according to Greengeenes (3, 16, 18)

Genus (phyllum)	The family	Examples
Firmicutes	*Clostridiaceae*	*Clostridium species*
	Ruminococcaceae	*Eubacterium species*
	Lachnospiraceae	*Fecalibacterium species*
	Streptococcaceae	*Lactobacillus johnsonii (P)*
	Lactobacillaceae	*Lactobacillus paracasei (P)*
	Bacillaceae	*Enterococcus faecium (P)*
	Erysipelotrichaceae	*Bacillus coagulans (P)*
Bacteroidetes	*Prevotellaceae*	*Prevotella species*
	Bacteriodaceae	*Bacteroides species*
Actinobacteria	*Coriobacteriaceae*	*Bifidobacterium longum (P)*
	Bifidobacteriaceae	*Bifidobacterium lactis (P)*
Proteobacteria	*Enterobacteriaceae*	*Escherichia coli*
	Sutterelaceae	*Salmonella enterica*
	Helicobactreiaceae	*Helicobacter pylori*
Fusobacteria	*Fusobacteriaceae*	*Fusobacterium motiferum*
		Fusobacterium perfoetens

There are marked differences in the phylogenetic composition of the gut microbiota between individuals of the same animal species and the metabolic products and end products are currently similar between animals. Similar members of the bacterial community are able to perform similar functions, and if a microbial group is missing due to disturbances (e.g. antibiotic therapy or other drugs), other members of the community are able to maintain stable system functions and take over the remaining uncovered roles (11, 14).

The commensal digestive flora is composed of a dense and complex layer of communities distributed throughout the digestive tract, and they can block the attachment and colonization of most enteric pathogens – this is known as competitive exclusion. Some commensal bacteria act by producing antibacterial substances such as surfactant, the compound that inhibits the growth and

development of pathogens. Viruses are also present at the digestive level, the so-called bacteriophages. Phage therapy, or bacteriophages, selectively targets some pathogenic bacteria to treat infections, but as a therapy, it has limited applicability at this time (11, 14).

The stable core microbiome in cats and dogs has not yet been fully defined, although there is more data in adult dogs compared to growing dogs. The definition of the stable central microbiome in growing puppies and kittens is due to interspecies and individual differences but also to the variability of the environmental factors in which they live and develop (3).

2. Synthesis and absorption of nutrients from food

The vast majority of colonic bacteria are anaerobic and their main functions are to produce energy from food and contribute to the competitive exclusion of potentially pathogenic bacteria. The slower the intake and the greater the availability of nutrients over time, the microbial diversity in the colon is favored (2, 15).

Bacteria in an ecosystem have discovered collaborative strategies to transform nutrient complexity for their benefit as well as that of the host. Bacteria in the colon provide digestive enzymes that allow complex carbohydrates to be used. Bacteria metabolize dehiscent epithelial cells, endogenous mucus, and undigested substances that have passed through the small intestine. Finally, digestive bacteria act by using nutrients normally present in the diet, metabolizing predominantly complex carbohydrates, including starch and dietary fibers such as cellulose, pectin, and fructans. Fermentation of these substances leads to the production of SCFAs - short-chain fatty acids (acetate, propionate, butyrate). Among his roles, the microbiota has proven to produce enzymes that facilitate the breakdown of food polysaccharides. It has also been shown that the microbiota through SCFAs can regulate intestinal blood flow, stimulate the growth and proliferation of enterocytes as well as mucin production along with influencing immune responses at the intestinal level. It also contributes to the metabolism of nitrates and proteins in food, which provides additional amino acids necessary for the processes of maintaining vital functions, reconstruction, and energy sources (2, 15, 16).

3. Digestive health maintenance

Digestive bacteria have the role of producing vitamins or converting some nutrients (fibers, proteins, lipids) or producing various metabolites such as short-chain fatty acids - SCFA, indoles, and secondary bile acids from primary bile acids, the production of neurotransmitters such as serotonin and gamma amino butyric acid (GABA). These metabolites fulfill roles such as maintenance of digestive motility, anti-inflammatory role, inhibit the development of enterotoxigenic bacteria, and improvement of the function of the intestinal barrier and the production of mucin layer, GABA – maintains healthy behaviors (1, 3, 15, 16, 18).

The simple movement of intestinal motility is a major defense mechanism against the attachment of pathogenic bacteria to the small intestine, and intestinal

motility below normal limits is often associated with dysbiosis. Physiological concentration of SCFAs stimulates intestinal motility in the canine ileum supporting the importance of microbial fermentation products in influencing host health (1, 9, 14).

The close relationship between gut microbiota and host cells is obvious, which will have a significant impact on gastrointestinal health. The causality of gastrointestinal diseases could be determined by transient pathogens at the digestive level, overgrowth with resistant opportunistic bacteria, or due to communication disturbances between the commensal flora and the innate immune system. The invasion of certain pathogens could profoundly disrupt the epithelium and intestinal functions by degrading the gastrointestinal mucosa (1, 9, 14).

A huge body of scientific evidence has shown that microbes play an important role in the pathogenesis of IBD (inflammatory bowel disease) in humans as well as in dogs and cats, with very similar mechanisms onset. Current theories regarding the occurrence of IBD favor the association between environmental factors, digestive microbiota, and the genetic susceptibility of the host, a fact demonstrated in Boxer dogs presenting with granulomatous colitis (1, 9, 14).

Similar to humans, studies have shown that feline and canine IBD is associated with immune imbalance, as differential cytokine expression has been identified in both small animal species with chronic enteropathies. The microbiota is involved in IBD in humans with inflammatory backgrounds present in the digestive segments with the richest number of bacteria. Studies in animal models of susceptibility to inflammation indicate that IBD only occurs if the bacteria are present. The cause-and-effect relationship between microbial imbalances and intestinal inflammation is not yet well established (13).

In recent years, the study of the microbiome continues to evaluate the roles of bacteria at the digestive level and the metabolites produced there, and diet can directly influence bacterial metabolites, and subsidiarily they can beneficially influence digestion but also organs such as the brain, skin or muscles (1, 4, 9, 12).

4. Supports immune function

The digestive tract has two major functions: the first is to absorb nutrients and defend the body against pathogens, thus a major role in supporting immunity. The immune function of the digestive tract is a significant priority as the digestive tract is the largest surface in the body that allocates space and immune responses and is continuously exposed to various organisms. The intestinal immune system is composed of the mucus layer in close interconnection with the epithelial cells, it secretes soluble immunoglobulin A and antimicrobial peptides. Tight junctions are interconnected parts of immunity (3, 8).

The mucus layer is composed of the outer layer where microorganisms can colonize and a compact inner layer that repels bacteria. A beneficial microbial community plays a key role in maintaining normal physiological homeostasis, but alteration of the immune system influences organ development and metabolism. As

a component of the intestinal immune system, the mucus layer prevents the adhesion of microorganisms to the intestinal epithelium and serves as the first protective barrier against infections. Maintaining the integrity of the gut, the junctions, and the basal cell layer is the body's next line of defense against pathogens (3, 8).

5. The relationship between digestion and brain health (gut-brain axis)

The impact of the digestive microbiome on the brain and emotional health has been extensively studied in both humans and dogs. The production of neurotransmitters such as serotonin and gamma amino butyric acid (GABA) is directly involved in these processes. In order to make the connections between the digestive microbiome and the behavior of the host, the phylogenetic profiles of the composition and structure of the digestive microbiome in dogs with aggressive, phobic tendencies were mapped and compared with those of normal behavior (2, 7, 15).

In agreement with the findings, behavioral disorders of aggression were characterized by an aberrant structure of the digestive microbiome, with a high biodiversity and richness of genera with generally subdominant bacteria (eg *Catenibacterium* and *Megamonas*). On the other hand, dogs with phobias were identified with flora richer in *Lactobacillus,* a bacterial genus known to have psychobiotic properties but which is generally known for its probiotic role (2, 7, 15).

Although further studies in this direction are needed, the current findings support the surprising possibility that some behavioral phenotypes in dogs may be associated with aberrant digestive microbiome profiles, suggesting possible connections between this aspect of dysbiosis and the central nervous system, thus indicating the likelihood of adopting probiotic therapies and with fibers aimed to restore host-microbiome balance to alleviate behavioral disturbances (2, 7, 15).

6. Relationship of the microbiome with the overweight and obesity onset

In recent years, multiple studies have been conducted to show the direct relationship between the body condition score - an expression of weight and physical health, revealing the obvious difference between metabolic, normal weight, overweight and obese profiles. The involvement of the microbiome in obesity is correlated with findings in mice, the lack of expression of the genes that code for leptin, whose products promote satiety. Obesity and even overweight are also characterized by disturbances in the composition of the microbiota - dysbiosis (6, 7, 11, 19).

In obesity, dysbiosis is the result of either the loss of key bacterial species or the reduction of the diversity of the digestive microbiome, or the overpopulation with potentially toxic bacterial species such as *Clostridium difficile* or *Bacteroides fragilis* (7, 11, 13).

In both humans and animals, changes in the abundance of predominant microbes have been associated with excess body fat through the presence of more *Firmicutes* and fewer *Bacteroidetes* (7, 11, 13).

Studying the gut microbiome of obese humans and animals has shown an increased abundance of certain species capable of extracting energy from complex polysaccharides (7, 11, 13).

The digestive microbiome of healthy dogs is co-dominated by three genera: *Fusobacterium, Bacteriodetes,* and *Firmicutes*, with a low proportion of Proteobacter and Actinobacter. In contrast to humans and other mammals, the microbiome of healthy dogs is more abundant in *Fusobacteria*. This taxonomic difference between normal individuals and obese animals may contribute to the development and perpetuation of obesity (6, 9, 17, 19, 20).

In obesity, nutrition has a crucial impact in changing the proportion and type of bacteria if the formula has a majorly different macro-nutritional profile, as is the case with high-protein, low-carbohydrate HPLC diets (10, 20).

Dysbiosis

When the imbalance of the digestive microbiome occurs as a result of various conditions or changes expressed by the numerical reduction of bacteria or their diversity, is called dysbiosis. This occurs secondary to the changes that occur at the digestive level through the influence of environmental factors or drugs such as antibiotic therapy, metronidazole, or tyrosine but also other drugs such as omeprazole. Since many of the bacteria cannot be cultured in the laboratory, the need for a standardized and accessible test to identify the presence of dysbiosis was identified, thus the Dysbiosis Index (DI) was created (2, 3, 7).

Recent research has established the dysbiosis index as a number (-1, 0, 2) and can be identified using dysbiosis tests available in Europe and North America, based on quantitative PCR measurement. The dysbiosis index 0-2 represents moderate changes in the microbiome, but the value >2 represents major changes in the digestive microflora (2, 3, 7, 15, 18).

The dysbiosis index is a predictor of the conversion of primary bile acids into secondary bile acids. The normal level of secondary bile acids has clinical importance for limiting the development of enterotoxigenic bacteria, thus secondary bile acids have an antimicrobial role (1, 2, 3).

The DI dysbiosis index, however, should be evaluated and interpreted in the context of the patient and the medical history, therapies, and condition and not as a single parameter (1, 2, 3).

Dysbiosis therapy

Dysbiosis is only one of the compounds of digestive diseases and multimodal therapy must address the primary cause. Although nutritional therapies, probiotics, prebiotics, symbiotics, and postbiotics are intensively explored, all these will be performed and adapted to each patient. In any case of digestive dysbiosis, it is recommended to restore microbiome balance, through different types of interventions, and depending on the severity of the dysbiosis and the time of the intervention, these could differ. Thus, in minor dysbiosis, it is helpful to simply change

the diet through nutritional intervention with diets containing hydrolyzed protein to limit undigested nutrients but also diets enriched with fiber, thus the main role of the diets is to moderate the digestive microbiome and restore the intestinal disorder. Depending on the type of fiber included in diets, these fibers will impact the growth of one or more bacterial genera present in the lumen (5, 12, 18).

The latest observations revealed that the simple inclusion of prebiotics or probiotics or a combination of both prebiotics and probiotics only has a beneficial impact for a limited period, only when administered, and the flora will return to the previous microbial population. When feed is enriched with prebiotic fiber, this approach is reliable and provides long-term health benefits, and contributes to the normalization of the microbiome (4, 14, 16, 17, 18). The therapeutic options in dysbiosis are summarized in Table 2 (18).

Recently, the crucial and defining role of more effective therapies by supplementing with dietary fiber and/or using therapeutic diets rich in fiber has been demonstrated (1, 18).

Only if these interventions are not effective, faecal microbiome transplantation (FMT) is recommended as a therapy customized according to the patient's needs (5, 15, 18).

Table 2

Therapeutic options in dysbiosis (18)

Treatment	Probable mechanism	Possible unwanted effects
Nutritional changes	*increased digestibility leads to a more limited substrate for bacterial overpopulation *elimination diets (with novel or hydrolyzed ingredients) remove antigens from food if the condition is immune-mediated	*none (if there is no food sensitivity present)
Prebiotic fibers	*growth of beneficial bacteria *prebiotics are converted to SCFA *fibers bind bacterial metabolites	*soluble/fermentable fiber can trigger flatulence initially
Probiotics	*can improve immunomodulation and digestive barrier function	*unwanted effects are rare, but it remains unclear which patient will benefit the most from which of the present strains

Antibiotics	*reduce the bacterial load and/or bacteria adhering to the digestive mucosa	*long-term changes in the microbiome *repopulation with the optimal microbiome when the antibiotic is no longer used * increased resistance to antibiotics
Fecal Microbiome Transplantation (FMT)	*enriches the microbiota in the lumen	*effectiveness depends on the primary disease, but side effects are rare *limited effect against bacteria adhering to the mucosa *recurrent dysbiosis when concurrent inflammation persists

Conclusions

The most recent studies consider the evaluation of the composition of the microbiota as well as its roles and impact at the digestive level, but also on the body as a whole, evaluating the contribution to maintaining the health of cats and dogs.

The digestive microbiome should be regarded as a metabolic organ with a major role in the development of the digestive tract and the support of some of the digestive functions, in maintaining health and directly or indirectly impacting multiple immune and metabolic functions with the role of producing energy, metabolites, and vitamins. The multiple metabolic pathways investigated and monitored proved their existence only due to the presence of bacteria in the digestive tract. Thus, the bacteria of the microbiota and the cells of the body should be seen as a whole rather than separate entities, as a "hidden organ".

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RETROSPECTIVE STUDY OF EXOCRINE PANCREATIC INSUFFICIENCY IN DOGS

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Summary

Exocrine pancreatic insufficiency (EPI) is a functional disturbance resulting from a lack of pancreatic enzymes. This condition occurs less frequently compared to pancreatitis and is mainly caused by atrophy of pancreatic acinar cells, followed by the malabsorption-maldigestion syndrome. Because of the high secretory capacity of the pancreas, signs of maldigestion are not seen until 90% of its secretory capacity is lost. The aim of the present study was to highlight clinical signs and laboratory findings that would assist in the early identification of EPI. The study consisted of an analysis of the medical records from 11 dogs diagnosed with EPI at the University Veterinary Clinics (CVU) from the Faculty of Veterinary Medicine, Timisoara. The diagnosis of this condition was based on history, clinical signs, and laboratory findings. Biochemical parameters, complete blood count, and urinalysis were performed for each patient by standard method. The highest prevalence was found in German Shepherds at ages between 1-4 years old. The majority of dogs diagnosed with EPI showed progressive weight loss and chronic diarrhea. The results of biochemical parameters values showed increased serum activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase, and decreased serum albumin concentration. The correlation between clinical signs and changes in serum biochemical parameters allows only the suspicion of exocrine pancreatic insufficiency, and for the confirmation of the diagnosis, it is necessary to measure Trypsin-like immunoreactivity (TLI).

Keywords: exocrine pancreatic insufficiency, German Shepherd, biochemical parameters.

Exocrine pancreatic insufficiency is a functional disturbance characterized by the insufficient synthesis of pancreatic enzymes. This condition occurs less frequently compared to pancreatitis (the most common form of exocrine pancreatopathy in dogs and cats, which eventually leads to EPI) and can be inherited (atrophy of pancreatic acinar cells, which is an autoimmune disorder) or acquired (secondary to chronic pancreatitis or pancreatic neoplasia) (11, 18, 19, 20).

The diagnosis of EPI, rather than pancreatitis, is based on clinical signs and laboratory findings. It is difficult to definitively identify the cause of clinical signs in EPI because specific diagnostic tests are needed, but pancreatic acinar atrophy is the most common cause (5, 12. 14, 18, 19).

Etiopathogenetic studies have shown that pancreatic acinar atrophy in German Shepherd and Collie dogs have some characteristics of immune diseases, indicating a genetic predisposition for this condition in these breeds. Other immunological studies in patients with partial pancreatic acinar atrophy have

suggested that both cellular and humoral immune responses play an important role in the pathogenesis of the condition, although tissue destruction appears to be mediated largely by cellular immune mechanisms (6, 7, 12, 18).

The decreased secretory capacity of the exocrine pancreas is the result of the progressive numerical reduction of acinar cells. The clinical effect is the development of severe maldigestion and malabsorption syndrome. Usually, the endocrine pancreas is not affected (5, 10, 18).

The reported clinical signs of EPI that are present in most affected dogs are polyphagia, progressive weight loss, increased fecal volume, poor digestion, flatulence, and chronic diarrhea. Less common clinical signs that are reported are eczema, poor coat, or skin disorder. The diagnosis of this disorder can only be suspected based on clinical signs and biochemical parameter changes, and for a positive diagnosis measuring the Trypsin-like immunoreactivity is necessary (1, 2, 7, 20).

The present study aimed to highlight clinical signs and laboratory findings that would assist in the early identification of EPI.

Materials and methods

The medical records of 11 dogs diagnosed with EPI at the University Veterinary Clinics from the Faculty of Veterinary Medicine, Timișoara were analyzed. The following data were registered for each patient: breed, age, gender, body condition score (BCS), clinical signs, as well as complete blood count (CBC) and serum biochemical parameters The ages of the dogs were between 1 and 11 years old. The BCS assessment was performed using a validated BCS, which is a 5-point rating scale with a range of categories from “very thin” to “obese”.

Blood tests were performed at the Laboratory of Functional and Metabolic Explorations at the University of Life Sciences, Timișoara. The CBC was determined by flow cytometry using an automatic hematology analyzer: (ProCyte Dx – IDEEX). The blood biochemical parameters, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total protein, creatinine, urea, cholesterol, and triglyceride were assessed by usually methods with an automated biochemistry analyzer (Rx Daytona^{+} -Randox). The serum TLI from all the dogs in this study was measured by radioimmunoassay and was less than 2.5 µg/L.

Results and discussions

Regarding the breed of the dogs diagnosed with exocrine pancreatic insufficiency, it was found that 54% of the dogs were German Shepherd, 27% were Half breed German Shepherd, 9% were Amstaff and 9% were German Shorthaired Pointer (Fig. 1). It should be noted that 81% of the dogs in this study were German Shepherd or half-breeds of this breed. Actually, in the literature, the highest

prevalence of EPI has been found in the German Shepherd breed (4, 8, 14, 15).

The majority of dogs in this study were diagnosed with exocrine pancreatic insufficiency at ages between 1 to 4 years (71%). An equal percentage (14%) of dogs aged between 5-7 years old and 8-11 years old were also diagnosed with the condition (Fig. 2). These findings are not surprising because exocrine pancreatic insufficiency develops over a long period before clinical signs appear, and a 90% of the pancreatic acinar destruction is required to occur EPI (4, 14, 18, 20).

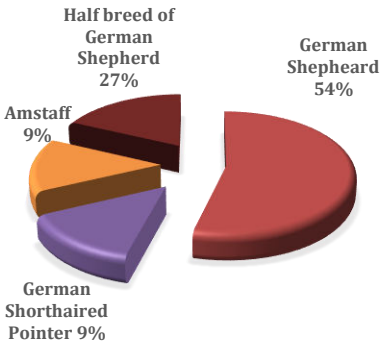

Fig. 1. The breed prevalence of dogs with EPI

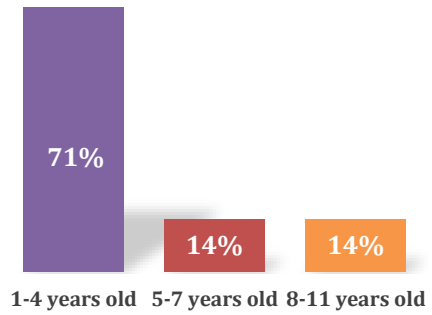

Fig. 2. The age prevalence in dogs with EPI

Assessment of body condition score (on a scale of 1 to 5) showed that 63% of the dogs were very thin (BCS 1), 27% were underweight (BCS 2) and only 10% had an ideal weight (BCS 3) at the time of consultation (Fig. 3). The loss of fat stores and muscle mass is due to negative energy balance resulting from insufficient absorption of lipids, proteins, and carbohydrate (16).

In this study, it was found that only 27% of the dogs diagnosed with EPI showed hypoproteinemia, and in the other 73%, total serum proteins were within normal limits (Fig. 4).

Serum albumin concentration was low in 63% of dogs with exocrine pancreatic insufficiency and only 37% had albuminemia within normal limits (3.0-4.7 g/dl) (Fig. 5). In general, hypoalbuminemia may be encountered in conditions that produce absorption and digestion disorders, in severe hepatic insufficiency and in diseases that lead to protein loss through diarrhea or urine (3, 16).

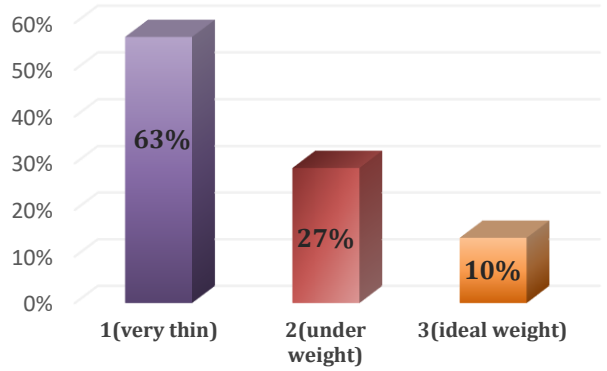

Fig. 3. Body Condition Score in dogs with EPI

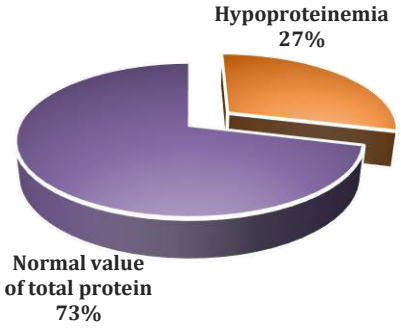

Fig. 4. Serum total protein concentrations in dogs with EPI

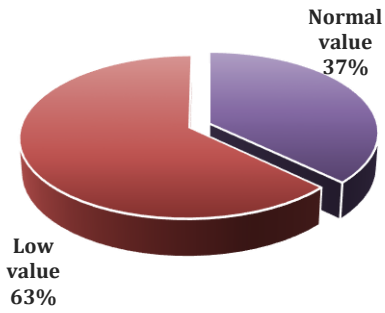

Fig. 5. Serum albumin concentration in dogs with EPI

Hypoalbuminemia in the dogs from this study was the consequence of maldigestion and malabsorption of protein, resulting from a deficiency of proteolytic enzymes that are secreted by the exocrine pancreas. The discrepancy between the high percentage of dogs with hypoalbuminemia and those with hypoproteinemia is due to the fact that total protein is the sum of serum albumin and globulin concentrations. Although hypoproteinemia is generally the consequence of hypoalbuminemia, serum total protein may record normal values as a consequence of increased globulin concentration even when serum albumin is low. The

percentage of dogs with hypoalbuminemia was equal to the percentage of dogs with BCS 1. As mentioned in the literature, decreased serum albumin concentration may be associated with loss of muscle mass (16).

Assessment of the serum activity of liver enzymes in dogs with exocrine EPI, a 2.5-fold increase over the normal value of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was found (Fig. 6). Also, biochemical findings revealed a significant increase of alkaline phosphatase (ALP). ALT and AST increases may be a consequence of hepatocellular injury due to the absorption of a greater amount of bacterial toxins from the intestinal tract into the portal circulation, due to intestinal bacterial overgrowth (19).

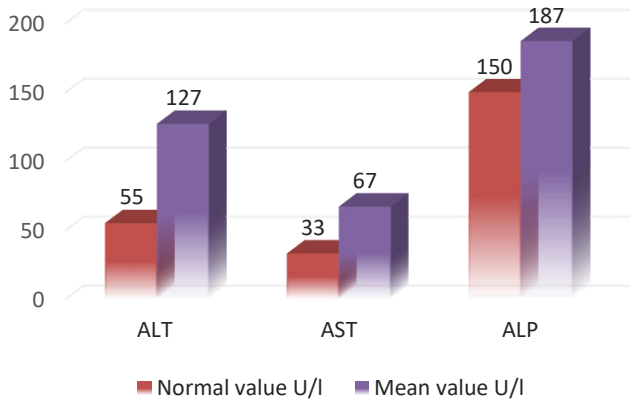

Fig. 6. Mean values of ALT, AST, ALP serum activity in dogs with EPI compared with normal mean values

The serum TLI measurements in all the dogs from this study were less than 2.5 µg/L. The literature describes that the trypsin immunoreactivity test is species-specific and pancreato-specific, and reference values for healthy dogs range from 5.7-45.2 µg/L. Values less than 2.5µg/L associated with typical clinical signs of maldigestion are considered to have high diagnostic value for severe exocrine pancreatic insufficiency and indicate a significant reduction in pancreatic digestive enzyme production (9, 11, 17).

Analysis of the complete blood counts of dogs with exocrine pancreatic insufficiency showed anemia in 43% of cases (Fig. 7). According to hematocrit and erythrocyte count values, anemia was mild in severity, and according to erythrocyte indices, anemia was normocytic and normochromic (13).

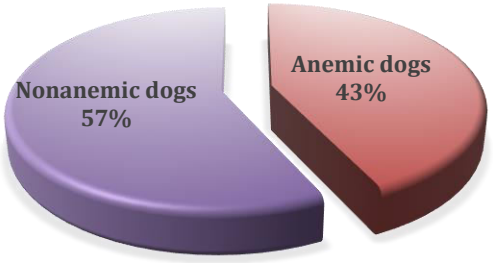

Fig. 7. The prevalence of anemia in dogs with EPI

Conclusions

In this study, the highest prevalence of EPI was recorded in the German Shepherd.

The most common clinical signs that may indicate EPI are weight loss, polyphagia, and chronic diarrhea.

Hypoalbuminemia along with increased liver transaminases are the most frequently altered biochemical parameters.

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THERMOGRAPHIC DIAGNOSIS OF THE INTERVERTEBRAL DISC DISEASE IN DOG: CASE REPORT

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Summary

Intervertebral disc disease (IVDD) represents a pathology that affects both people and animals. In dogs, the disease is painful and evolves clinically with pain, loss of motor reflexes, tetraplegia, or paraplegia of the hindlimbs. The gold standard diagnostic methods are represented by Magnetic Resonance Imaging (MRI) and Computed Tomography (CT scan). In particular situations, is required to inject a contrast solution Ultravist 370 (370 mg/ml) Bayer in the subarachnoidian space, a procedure which involves risks to the spinal cord. Both of these diagnosis methods demand sedation, and exposure of the dogs to radiation and are time-consuming and costly. Thermography is an imaging diagnostic method that highlights an area with inflammation and will save money, and time and reduce the risk of spinal cord injury and radiation exposure. This case report used the FlirE50 to scan the thoracolumbar region in a patient diagnosed with IVDD after radiography and CT scan with contrast solution. The site of inflammation identified after thermographic evaluation of the thoracolumbar area corresponds to the same space as IVDD. Thermography can be used as a complementary method to diagnose IVDD in dogs based on the inflammatory reaction which resulted after disc trauma.

Keywords: dog, intervertebral disc disease, thermography.

Imaging is integral in the diagnosis of intervertebral disc disease (IVDD) in dogs. Many imaging techniques have been described in dogs such as radiography, myelography, computed tomography (CT), and magnetic resonance imaging (MRI). Intervertebral disc disease (IVDD) is a common cause of neurologic dysfunction in dogs (4), so a thorough understanding of the indications and limitations of each imaging modality to aid in the diagnosis, treatment planning, and the prognosis is essential to successful case management (11).

The first report of a case with IVDD was described by Dexler in 1896 in a Dachshund, and the persons who truly well-characterized were Olsson and Hansen in 1950 (14). Hansen classified IVDD in acute disease - Hansen type I intervertebral disc extrusion (IVDE), and chronic - Hansen type II intervertebral disc protrusion (IVDP) (9). After the introduction of MRI in the diagnosis of IVDD, new types of lesions were identified, such as acute non-compressive nucleus pulposus extrusion (ANNPE), hydrated nucleus pulposus extrusion (HNPE), and intradural/intramedullary intervertebral disc extrusion (IIVDE) (10).

Intervertebral disc extrusion is the most commonly described clinical form of intervertebral disc herniation (IVDH), which leads to acute spinal pain, unilateral hind limb lameness, or paraplegia of the rear train (13). Radiography was the most used

method for the diagnosis of IVDD starting in the 1950s and extending until 1980 (5). The use of general anesthesia has been recommended in order to obtain diagnostic radiographs. The radiographic sign for IVDE is the presence of the mineralized disc material in the vertebral canal and vacuum phenomenon, increased opacity of the intervertebral foramen, and narrowing of the disc space (2).

Myelography started to be used in 1970, but the first publication with this method of diagnosis appeared in the middle of 1990 (16). Myelography is an invasive procedure and presents some risks, primarily temporary deterioration of the neurologic status, and post-myelography seizures (6). The sign for disc extrusion in myelography is represented by the absence of the contrast line at the site of the extrusion (12).

Computed tomography is an efficient method for diagnosing IVDD in dogs (16). There exist 3 ways to achieve a diagnosis of IVDE using CT: conventional CT, intravenous contrast CT (CT-angiography), and subarachnoid contrast CT (CT-myelography) (3, 5). CT scan has certain advantages compared to MRI, including a lower cost and much faster scan time. For dogs in which conventional CT alone is insufficient, CT myelography can be utilized for a diagnosis. Signs for IVDD after a CT scan are represented by acute or chronic mineralized nucleus pulposus, vacuum in the disc material. CT-myelography represents a superior method to diagnose acute extrusion when compared to conventional CT scans (18).

MRI is considered the gold standard for diagnosis the of IVDD in humans and companion animals. Compared to a CT scan, an MRI scan does not require subarachnoid contrast medium administration because of the ability to alter tissue contrast by applying different acquisition sequences, so the risks associated with myelography are avoided (5). The findings on MRI scans consist of edema, gliosis, and malacia of the spine (7).

Medical infrared imaging or thermography is a non-invasive imaging method used to detect abnormal physiologic changes in people and animals (15, 20). Areas with increased temperature in local circulation and metabolic rate can be clinically associated with inflammation, and trauma, as compared to regions of decreased temperature that can be associated with decreased tissue perfusion secondary to a vascular shunt, infarction, or changes in the autonomic nervous system (8).

Materials and methods

A 5-year-old female Dachshund was admitted to the University Veterinary Clinic from the Faculty of Veterinary Medicine from Timişoara for evaluation of severe paraplegia (Fig. 1). At the time of presentation for the clinical examination, the dog presented paraplegia on the hindlimbs of 1-day duration. This was the first episode of paraplegia in her life. The owner did not visit another clinic before coming to us and the animal did not receive any medical treatment.

Fig. 1. Dachshund female with paraplegia.

Was performed the clinical and neurological examinations. During clinical examination, rectal temperature was measured, the dehydration stage was appreciated, and palpation of the abdomen and the hindlimb muscles was performed.

For the neurological examination, we took into consideration the withdrawal reflex, proprioception, signs of paraplegia, anal sphincter reflex, and patellar reflex.

The hair in the thoracolumbar region was clipped once a doubt of disc herniation was established in this region. By using the FlirE50 device, thermography of the region was performed after a period of accommodation of 30 minutes with the ambient temperature.

Radiographs and CT scans of the thoracolumbar area were performed to establish the diagnosis. For this purpose, to be able to achieve the imaging examinations, the patient was sedated using Diazepam 0.5% (0.4 mg/kg), Ketamine 10% (8 mg/kg) and supplemented with Propofol 1% (3-7 mg/kg).

To obtain the radiographic images, we used a Siemens Multix Swing machine, and two views of the thoracolumbar area were obtained – latero-lateral and ventro-dorsal.

A Siemens Somatom Definition AS 64 CT scanner was used to perform the scan and allowed us to obtain multiplanar sections (MPR) with a dimension of 1 mm. Furthermore, a contrast solution of Ultravist 370 (370 mg/ml) ® Bayer was administered in the subarachnoidian space for proper evaluation of spinal cord compression in case of acute disc herniation.

Results and discussions

At the clinical examination, the patient presented a rectal temperature of 38.4°C, urinary incontinence, abdominal pain, no muscular amyotrophy of the hindlimbs, and no dehydration.

A neurological examination of the patient revealed a positive response to deep and superficial pain in the hindlimbs, a negative response to proprioception, a positive reaction to the anal sphincter reflex, and a diminished response to the patellar reflex.

During the thermographic examination of the thoracolumbar space was identified a warmer area centered in the middle of the lumbar area, corresponding with the intervertebral space L_4-L_5 (Fig. 2).

At the radiological investigation, in the latero-lateral view was observed narrowing of the articular facets and spondylosis of the intervertebral space L_4-L_5 (Fig. 3) and in the ventro-dorsal survey was observed narrowing and wedging of the L_4-L_5 intervertebral space.

The results obtained after performing the CT scan showed a narrowing of the intervertebral space L_4-L_5 and early mineralization in the intervertebral disc without extruded material in the vertebral canal (Fig. 4). After performing the CT scan with contrast medium, was observed a deviation of the contrast column which suggests an extradural compression of the spine produced by an acute disc extrusion in the L_4-L_5 intervertebral space (Fig. 5).

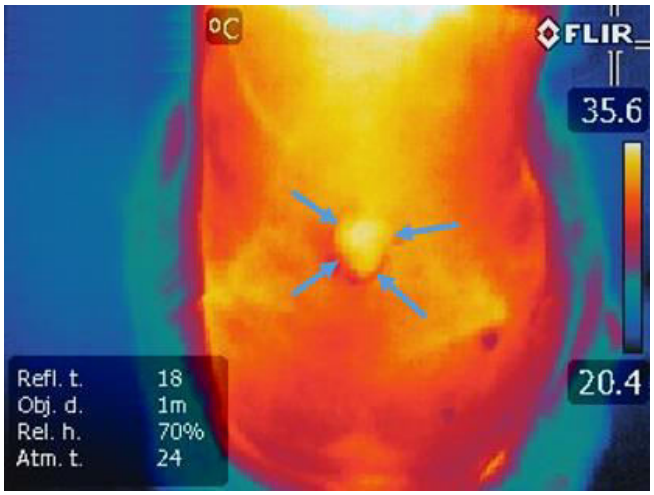

Fig. 2. Thermography of the lumbar area. Increased temperature “hot spot” in the L_4-L_5 area (blue arrows)

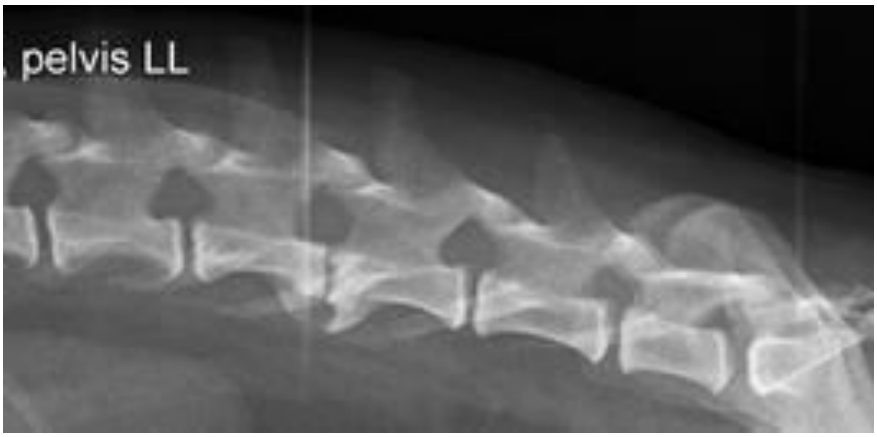

Fig. 3. Narrowing of the articular facets and spondylosis of the intervertebral space L_4-L_5

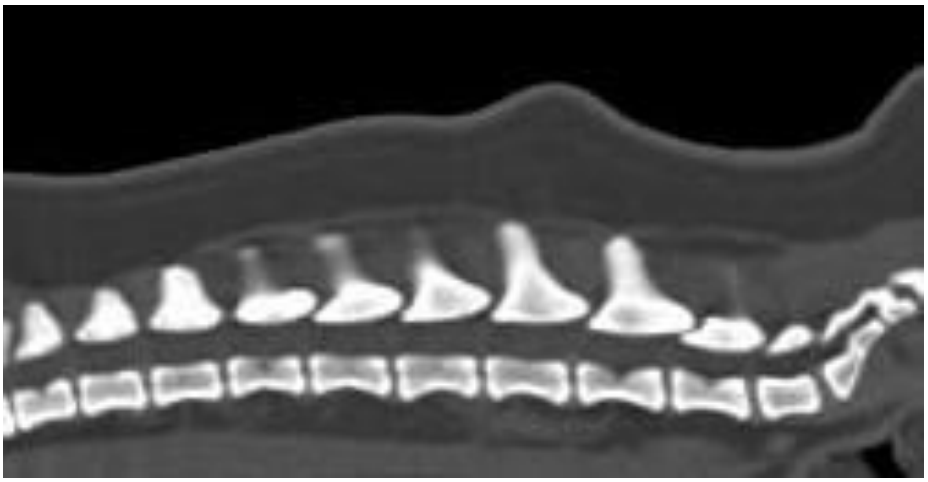

Fig. 4. Narrowing of the intervertebral space L_4-L_5 and early mineralization in the intervertebral disc

In this study, we investigated the use of thermography as a method of diagnosis the intervertebral disc disease. The diagnosis of IVDD was confirmed after radiological, conventional CT, and after CT-myelography.

Postmortem studies suggested that Hansen type I extrusions occur more commonly in chondrodystrophic breeds (Dachshund, Pekingeses, French Bulldogs, Basset Hounds) and Hansen type II protrusions in nonchondrodystrophic breeds (4).

IVD herniation is rare before the age of 2 years and appears more frequently between the ages of 3 and 7 in chondrodystrophic patients.

Fig. 5. Deviation of the contrast column suggesting an extradural compression of the spinal cord

The radiological examination reveals in the case of IVDD narrowing of the intervertebral space and increased opacity of the intervertebral foramen on latero-lateral views, and narrowing of the intervertebral space and in the same case disc mineralization in the intervertebral space in ventro-dorsal view (19).

In the past, myelography was the main imaging diagnostic method for disc herniation in animals and is still used nowadays. At present, a decision to use myelography for a dog suspected of acute disc herniation is usually based on the lack of availability of either CT or MRI, but includes some complications such as invasiveness, which can cause spinal cord damage, and holds potential for adverse reactions to contrast medium (16).

CT is an accurate diagnostic imaging modality for dogs with disc herniation. CT imaging after administration of the contrast medium in the subarachnoid space combines the benefits of CT and myelography (16).

Conventional CT identified a small amount of disc material between L_4-L_5, but the CT myelography revealed an important compression and dorsal deviation of the contrast line in the intervertebral space L_4-L_5. The demonstration of mild spinal cord compression in chondrodystrophic dogs using CT may indicate that decompression of these dogs is not necessary, but the administration of contrast

medium in the subarachnoid space will elucidate the necessity of decompression (13).

Thermography records the emission of surface heat from the skin and generates thermal patterns in the form of a color map. Regions of elevated temperature are related to an increase in local circulation and metabolic rate and can be clinically associated with inflammation (11, 17).

The thermographic images strongly correlate with MRI findings in the case of IVDD in dogs. Thermography can be used as a diagnostic method to reduce the overall cost when compared to MRI and CT diagnostic methods, where the costs are increased (1, 17).

In dogs with IVDD, we observed a “white spot” in the region at the level of the herniated intervertebral disk. The affected intervertebral space was represented by a focal area of hyperthermia as was reported in horses with neurologic injury (8, 20).

Conclusions

In intervertebral disc disease, the affected intervertebral space presents a warmer area compared with healthy ones. The thermal camera FlirE50 can detect the changes in local temperature in case of intervertebral disc disease.

Thermography’s a noninvasive, cheap, and risk-free method for diagnosing intervertebral disc disease in dogs and can be used as a complementary imaging method in this regard.

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