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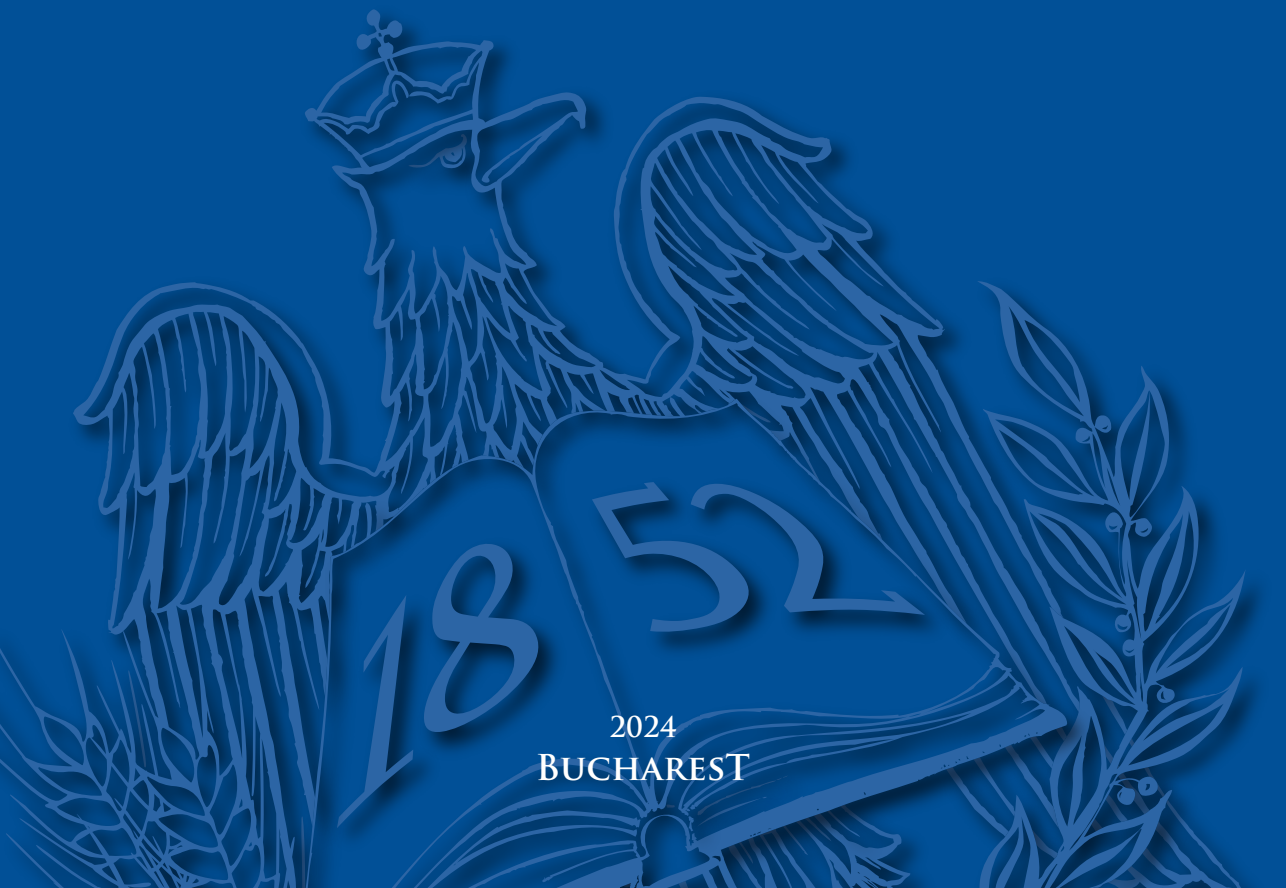

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SUMMARY

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STUDY ON THE MORPHO-TOPOGRAPHY AND VASCULARIZATION OF THE PAROTID GLAND IN SMALL RUMINANTS

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Abstract

The parotid gland is one of the major salivary glands present in domestic mammals, mainly developed in herbivores. In the specialized literature, there are studies related to the size and development of the gland in ruminants, analyzing correlations between diet and parotid gland morphometry. The parotid region is an area of interest for clinicians, given the traumatic lesions or tumor formations that can occur in this region and which also lead to pathologies of the large salivary glands. Despite the low incidence of salivary gland diseases in most domestic animal species, inflammatory processes may occur, and medicamentous treatment may be associated with surgical treatment. The study focused on aspects of the morpho-topography and vascularization of the parotid gland in small ruminants, also describing its rapport with vascular-nervous and lymphatic structures. Among the most important differences and individual variations observed are differences in gland morphometry and variations in the topography of the parotid duct.

Key words*: parotid duct, parotid gland, small ruminants.*

INTRODUCTION

The saliva is the result of the secretion of the agglomerated and disseminated salivary glands. The most developed gland in sheep is the parotid gland, which has a single parotid duct (Uzun et al., 2022). Even though salivary gland pathologies are rare in domestic mammals, their symptomatology is severe. It can affect the animal's general condition, dysphagia and inappetence being the main symptoms.

The parotid region in humans has also been studied since the 19th century, considering the benign or malignant tumor processes that can affect the parotid gland (Burns, 1823). Various studies have presented in detail: the parotid fascia, the relationship of the gland to the vascular-nervous structures, and the importance of identifying the facial nerve and its branches when performing surgery in the parotid area (Carr, 1984; Gaughran, 1961; Furstenberg, 1941). There is great interest in understanding the relationships between the constituent formations of this region. The pathologies related to the salivary glands are diverse and may include various conditions, such as trauma, fistulae, inflammation, and neoplastic processes. In the case of benign or malignant tumors at this level, excision of the parotid gland is necessary. A complication of parotidectomy, the procedure used for parotid gland tumors, is an injury to the facial nerve, and preserving its integrity requires special attention (Salih et al., 2022; Gaillard et al., 2005).

There are few scientific studies on the morphology and pathology of salivary glands in livestock, although traumatic injuries involving the cervical-cephalic region are common in these species. In contrast, there are studies on sheep that address the lympho-nodular formations in this region, but also the venous vasculature, more specifically, the distribution of the external jugular vein (Georgescu et al., 2010; Predoi et al., 2011; Predoi & Belu, 2001). Due to the numerous vascular and nervous formations whose path intersects the parotid region, interventions in this area require a good knowledge of the macroscopic morphology of the region.

MATERIALS AND METHODS

The study selected two ruminant species for investigation, namely domestic sheep (*Ovis*

aries) and roe deer (*Capreolus capreolus*). Despite belonging to different families, *Bovidae* and *Cervidae,* respectively, these ruminants share similar anatomical characteristics. The study material comprised anatomical parts from four sheep and two roe deer heads, all of which were adults. The roe deer heads were obtained from hunting grounds in Buzău County, while the domestic ruminant heads were sourced from farms.

The detachment of the head from the trunk was realized at the C5-C6 joint level to maintain the integrity of the regional vascular-nervous structures. First, the vessels were flushed with 0.9% NaCl saline before injection of a natural rubber-based substance to facilitate better distribution. The arteries and veins were injected with different staining before skinning to enhance visualization. The anatomical parts were kept at -10°C for 24 hours to allow for the solidification of the liquid latex.

The dissection was performed in an organized manner, starting with the removal of the skin from the head region. The initial step involved the removal of the parotid fascia and the parotido-auricular muscle, which led to the exposure of the parotid gland. Subsequently, the gland's relationships with the vascular, nervous, and lymphatic formations were closely observed. In individuals where the lumen of the duct was large enough, the same substance used for vascular structures was injected. The Nomina Anatomica Veterinaria - 2017 was referred to identify and describe the anatomical structures.

RESULTS AND DISCUSSIONS

The research conducted by Hoffman (1989) on the digestive systems of 65 ruminant species from the four continents allowed their classification according to diet into concentrate selectors, intermediate (mixed) feeders and grass and roughage eaters. In the present research, we have choosen two species with distinct diets. Roe deer feed mainly on leaves of trees and shrubs, alfalfa, and clover and are considered "browsers", unlike domestic sheep, which eat mainly grass, fibrous, concentrated feed and are called "grazers".

The information on domestic ruminants cannot be generalized to wild species.

Following the research carried out in **sheep**, the dorsal extremity of **the parotid gland** (*Gl. parotis*) extends only cranially from the base of the auricular cartilage without extending beyond the level of the zygomatic arch. There is no retroauricular angle. (Figures 1, 2). The preauricular angle of the gland is located between the auricular and palpebral branches of the terminals of the rostral auricular artery and is irrigated by 2-3 thin branches of the auricular branch. This portion is in turn divided into two processes: the rostral one is irrigated by the palpebral branch and the posterior one receives branches from the auricular branch. The two processes are arranged on either side of the palpebral branch.

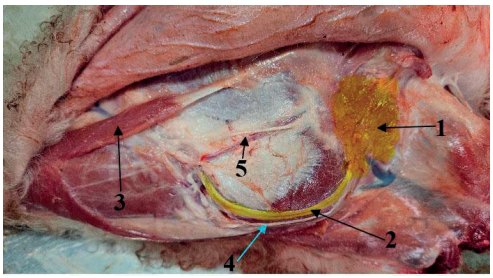

Figure 1. Parotid gland in sheep:
1 - the parotid gland; 2 - the parotid duct; 3 - zygomatic muscle; 4 - ventral buccal branch of the facial nerve; 5 - dorsal buccal branch of the facial nerve

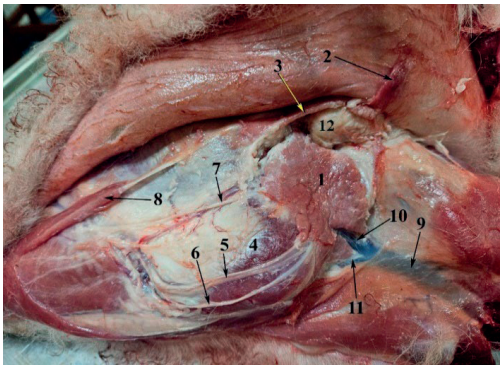

Figure 2. Parotid gland in sheep in relation to vascular-nervous formations:
1 - the parotid gland; 2 - the parotido-auricular muscle; 3 - the zygomaticoauricular muscle; 4 - the masseter muscle; 5 - the parotid canal; 6 - ventral buccal branch; 7 - dorsal buccal branch; 8 - the zygomatic muscle; 9 - external jugular vein; 10 - maxillary vein; 11 - linguofacial vein; 12 - auricular cartilage

The parotid duct (*Ductus parotideus*) arises from the ventral extremity of the gland, crossing

the middle third of the lateral surface of the masseter muscle, and opens through a reduced parotid papilla to the right of the superior first molar. This duct has an average length of 14.5 cm (Figure 3).

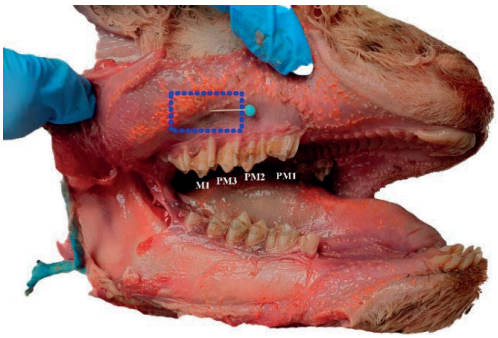

Figure 3. Topography of the opening of the parotid duct in sheep: PM1, PM2,PM3 - premolars; M1 - molar

The rostral auricular nerve, a branch of the facial nerve, emerges before the division of the facial nerve into the dorsal and ventral buccal branches and is located immediately behind the annular cartilage, deep to the gland, about 1 cm deep. The dorsal buccal nerve crosses the gland in a plane that passes midway along the parotid gland's longitudinal axis, about 3 cm from its upper end. The ventral buccal branch runs towards the origin of the gland duct, being located intra-acinar, with an oblique disposition towards the base of the ear (Figure 4).

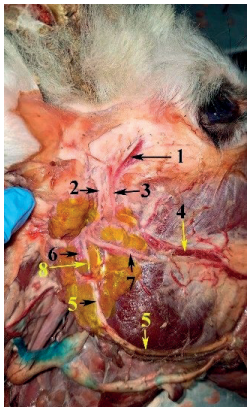

Figure 4. Topography of vascular-nervous structures in relation to the parotid gland in sheep (deep layer):
1 - rostral auricular artery; 2 - rostral auricular nerve;
3 - rostral auricular vein; 4 - transverse facial artery;
5 - parotid duct; 6 - caudal auricular artery; 7 - dorsal buccal branch; 8 - ventral buccal branch

The parotid lymphocenter is entirely or partially covered by the parotid gland, with an average length of 12 mm and an average width of 7 mm. It covers the terminal portion of the aurico-palpebral vein and is irrigated by branches from the transverse facial artery. In one specimen, the parotid lymphocenter was represented by two lymph nodes. The first is located on the surface of the masseter muscle, covering the origin of the transverse facial artery in contact with the inferior border of the dorsal buccal branch. The second, more reduced one is placed caudal of the terminal portion of the anterior auricular vein in relation to the rostral margin of the parotid.

Regarding the arterial vasculature of the parotid gland, a voluminous division branching off at the origin of the transverse facial artery descends obliquely in the middle third of the caudal margin of the masseter muscle and gives along the intraglandular course parotid branches (4-5). Opposite to this voluminous branch, also branching off from the transverse facial artery, are direct dorsal glandular branches or muscle branches, which also indirectly have glandular branches (Figure 5).

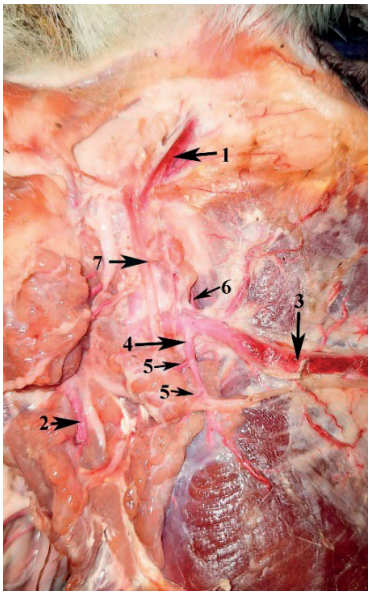

Figure 5. Transverse facial artery in sheep:
1 - rostral auricular artery; 2 - caudal auricular artery;
3 - transverse facial artery; 4 - ventral masseteric branch;
5 - glandular branches arising from the masseteric branch; 6 - dorsal masseteric branch;
7 - rostral auricular vein

The rostral edge of the gland covers the origin of the transverse facial artery from which it receives fine branches.
The dorsocaudal margin of the gland is served by the caudal auricular artery. The ventral extremity does not extend beyond the linguofacial vein. It is supplied with the portion below the origin of the parotid duct by a retrograde branch of the lingual artery parallel to the linguofacial vein.
At the origin of the caudal auricular artery arises the most important parotid branch for the middle portion of the body. This branch is directed ventro-laterally above the anterior belly of the digastric muscle.
In the **roe-deer**, **the parotid gland** forms at the dorsal extremity two angles, pre- and retro auricular; the anterior edge of the gland is convex, being delimited by the vertical branch of the mandible, and the caudal auricular vein delimits the caudal edge. The gland's ventral edge reaches the sterno-zygomatic muscle's upper edge (Figure 6).

Figure 6. The parotid gland of *Capreolus capreolus*: 1 - auricular cartilage; 2 - parotid lymphocenter; 3 - masseter muscle; 4 - external jugular vein; 5 - linguo-facial vein; 6 - parotid gland; 7 - parotid duct; 8 - facial artery; 9 - parotido-auricular muscle; 10 - maxillary vein; 11 - rostral auricular artery

Notably, a distinct triangular-shaped gland lobe connected by a narrow dorsal glandular part located on the lateral aspect of the linguofacial vein was observed. This portion is vascularized by a branch branching from the common carotid artery, the branch terminating in the sterno-zygomatic muscle. Its most caudal angle is at the level of a transverse plane passing through the caudal edge of the atlas wing.

The parotid gland has a maximum length of 10 cm and a maximum width of 4 cm below the base of the auricular cartilage.
Unlike the domestic ruminant, in *Capreolus capreolus*, the parotid lymphocenter is not covered by the parotid gland and is visible after the parotid fascia is removed. A single lymph node formation was observed in both specimens, approximately 2.5 cm long and 1 cm wide.
The facial artery (absent in sheep) gives off an inferior labial artery lying superficially at the ventral edge of the buccinator muscle, straight and then 3-4 branches in a caudal direction on the surface of the masseter. The artery also gives off a buccal branch and an artery corresponding to the angular artery. The parotid duct is detached from the ventral extremity of the dorsal portion opposite the maxillary vein of the gland. This duct follows the medial pterygoid muscle's medial border about 1 cm dorsal to the ventral border of the mandible. It runs up to the vascular notch and exits into the facial plane. The duct follows the facial vein in the inferior third of the masseter margin at its deep surface, the two formations flanked cranially by the facial artery. The duct remains covered by the facial vein for most of its length, up to the level of the upper dental arch where it runs sharply rostral. It then perforates the buccinator muscle and opens at the level of the upper first molar, the parotid papilla being inconspicuous. This duct is approximately 23.5 cm long (Figures 7, 8).

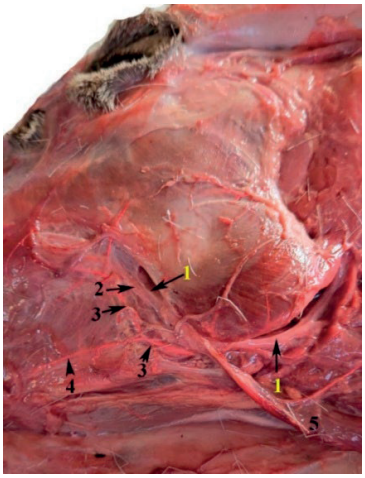

Figure 7. The pathway of the parotid duct in roe- deer: 1 - parotid duct; 2 - facial vein; 3 - facial artery; 4 - inferior labial artery; 5 - sterno-zygomatic muscle

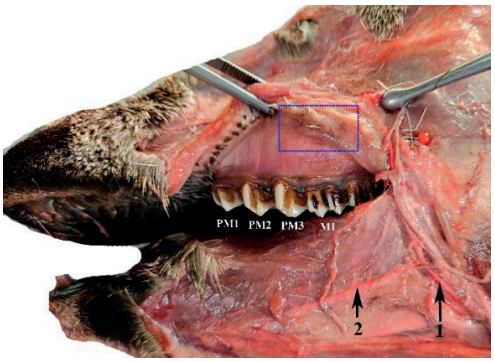

Figure 8. The opening of the parotid duct in *Capreolus capreolus* (after horizontal sectioning of the buccinator muscle):
PM1, PM2,PM3 - premolars, M1 - molar 1;
1- facial artery; 2- inferior labial artery

The table below contains an average of the results obtained from the study, the weight of the animals was measured after slaughter, taking into account the weight of the digestive tract, the content of which was subsequently subtracted (Table 1).

Table 1. Parotid gland

Species	Length	Width	Weight	% of the body mass
Ovis aries	47 mm	23 mm	13 g	0.04%
Capreolus capreolus	100 mm	41 mm	15 g	0.1%

CONCLUSIONS

One particular aspect was the differences in gland development between the two species. Measurements showed that the parotid gland of the roe deer has an average weight of 15 g and represents about 0.1% of the animal's body mass. Compared to the domestic ruminant, where the parotid gland although having a similar average weight of 14 g, represents 0.04%. This difference is the result of the diet of the two species. The deer is a selective species in terms of the plants consumed, choosing the foods with the highest nutritional value, and the resting periods between meals are short, thus requiring the production of a greater quantity of saliva to buffer the ruminal pH to ensure proper digestion.

Regarding the parotid duct, it has a distinct pathway in the two species. In domestic sheep, the duct is shorter, following the lateral surface of the masseter muscle in its inferior third. The duct comes into contact with the dorsal margin of the ventral buccal nerve. In *Capreolus capreolus*, the pathway of the parotid duct is similar to that in cattle, passing initially at the medial aspect of the mandible before returning superficially to the level of the vascular notch. The vascular formations maintain their relationship to the parotid duct along the rostral margin of the masseter muscle. The facial artery lies rostrally, followed by the facial vein, and the duct is located aboral and deep to the vein.
In the individuals examined in the study, regardless of species, the parotid duct opened at the level of the first upper molar.
Salivary glands in ruminants represent a subject of further interest for other studies involving wild and domestic species.

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MORPHOLOGICAL PARTICULARITIES OF THE JACKAL SKULL (*CANIS AUREUS*) - CASE STUDY

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Abstract

This study aims to describe the morphological characteristics of the jackal skull. The jackal (Canis aureus) belongs to the Order Carnivora, Family Canidae, and is widespread in northern Africa and southern Europe. In Romania, the number of specimens of this species has dramatically increased in recent years because the jackal has no natural predators, and wolf specimens are rare. The skull features are valuable elements of species identification. Data on skull morphology in jackals in the specialized literature are few and lacking in detail. A skull from an adult specimen from the Anatomy discipline collection was used for the study. The following conclusions emerged: the external sagittal crest is very high; the paracondylar processes are attached to the tympanic bulla; the foramen lacerum is absent; the presence of 2-3 lesser palatine foramina, placed caudal to the greater palatine foramen; the supraorbital foramen is absent; on the medial face of the angular process there is a reduced fossa.

Key words*:jackal, lesser palatine foramina, skul.*

INTRODUCTION

The jackal (*Canis aureus*) is part of the *Carnivora* family, presenting phenotypic and genotypic characteristics similar to those of the wolf and coyote.

There are numerous studies in the specialized literature, some on the jackal's dentition, which noted differences compared to wolves; the canines are thinner but more robust, while in the carnivores, they are weaker. All differences are correlated with specific feeding characteristics. Similarities to the domestic dog have been observed; the dentition is diphyodont, heterodont, brachydont and secodont type, and the upper dental arch is longer than the lower one (Stan, 2016).

Macro-anatomical studies were conducted on the golden jackal skull to achieve clinical applications during anesthesia (Louei Monfared, 2013).

Sexual dimorphism in golden jackals, considering the linear body parameters and weight between males and females, is weaker than in red foxes and wolves (Raichev et al., 2017). There are several studies on head variability and differences in morphometric data of jackal skulls among jackal species found in Europe, Asia and Africa (Stoyanov, 2020; Markov et al., 2017; Rutkowski et al., 2015).

There are various studies on other carnivores' skull morphology, but mostly on felines such cheetas, and domestic cats and a few on other skull morphology such as brown bears (Roșu et al., 2016; Georgescu et al., 2016; Roșu et al., 2022).

MATERIALS AND METHODS

A skull from an adult jackal (*Canis aureus*) was used for the study. The skull belongs to the collection of Anatomy discipline of the Faculty of Veterinary Medicine Bucharest. The most relevant morphological aspects were described and photographed. Description and identification follow the Nomina Anatomica Veterinaria (N.A.V.), 2017.

RESULTS AND DISCUSSIONS

The jackal's skull (*Canis aureus*) is of the dolichocephalic type, with an elongated and narrow appearance.

The dorsal side of the skull has an elongated and narrow appearance, with a maximum

neurocranium width in the middle portion of the parietal bones.

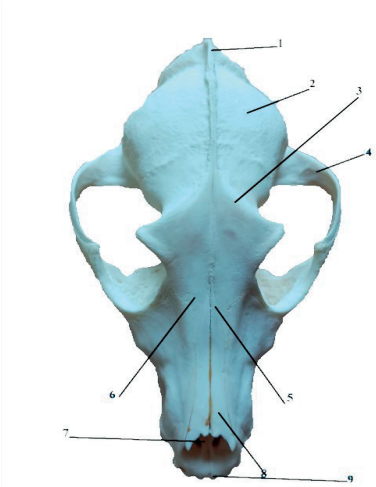

Figure 1. Dorsal face of jackal (*Canis aureus)* skull – original:
1. External sagittal crest; 2. Parietal; 3. Temporal line; 4. Zygomatic process of the temporal; 5. Frontal process of the nasal; 6. Nasal process of the frontal; 7. Nasal notch; 8. Nasal; 9. Interincisive foramen

The external sagittal crest starts from the external occipital protuberance, is very high at the interparietal bone level and in the parietals' aboral extremity, and decreases in a rostral direction. At the frontal level, it divides into two temporal lines (Figure 1).

The frontal bone presents, rostrally, a nasal process between the caudal extremities of the nasal and the maxilla.

The zygomatic processes of the frontals are very short, directed ventro-laterally and lack supraorbital foramina at the base. The dorsal surface of the frontals is slightly convex, creating a small, narrow and slightly elongated frontal fossa at the level of the interfrontal suture.

The nasals are narrow and elongated, ending in the caudal extremity through very sharp frontal processes. The nasal bone ends bifid at the rostral extremity, creating a wide notch between the short medial and elongated lateral parts (Figure 2).

The nasal process of the incisive bones ends tapered, exceeding the maxilla's upper half edge, and the body of the incisive is convex in the caudo-rostral direction.

The vomer presents a deep septal groove, and in its caudal extremity, it does not articulate with the aboral portion of the palatine processes, delimiting a vast subvomeral space through which the two nasal cavities communicate.

The nasal opening is wide, so the single first endoturbinate and the divided maxilloturbinate are visible.

Figure 2. The rostral extremity of the jackal (*Canis aureus*) viscerocranium - original
1. Canine fossa; 2. Nasal; 3. Maxilloturbinate; 4. Vomer; 5. Incisive; 6. Maxilla; 7. Infraorbital foramen

At the rostral extremity of the interincisive suture there is a very narrow interincisive canal visible on both sides.

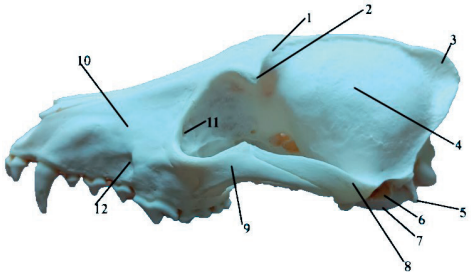

Figure 3. Lateral view of jackal (*Canis aureus*) skull – original:
1. Temporal line; 2. Zygomatic process of the frontal; 3. External sagittal crest; 4. Parietal; 5. Jugular process; 6. External acoustic meatus; 7. Tympanic bulla; 8. The zygomatic process of the temporal; 9. Zygomatic; 10. Maxilla; 11. Lacrimal foramen; 12. Infraorbital foramen

The orbit is incomplete (Figure 3). The convex temporal fossa communicates broadly with the orbit in a rostral direction. The orbito-temporal crest is short but very well-defined in its dorsal

extremity. The temporal line of the zygomatic bone is well-highlighted.
The fossa of the lacrimal sac is narrow and has a single lacrimal foramen. At the level of the maxilla, there is a large, elongated infraorbital foramen located caudal to the reduced canine fossa. The maxillofacial crest is absent.

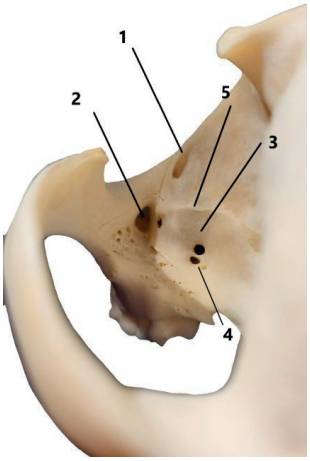

Figure 4. Maxillary hiatus (*Canis aureus*) - original:
1. Lacrimal foramen: Maxillary hiatus: 2. Maxillary foramen; 3. Sphenopalatine foramen; 4. Caudal palatine foramen. 5. Inferior orbital crest

On the medial wall of the orbit is a reduced inferior orbital crest that descends from the level of the lacrimal foramen. Caudally, above it, is an ethmoid foramen. The supraorbital margin is reduced in length and does not show a supraorbital notch or foramina.
The maxillary tuberosity features a reduced medio-caudal spine toward the pterygopalatine crest. Within the maxillary hiatus there are three foramina: maxillary, sphenopalatine, and caudal palatine. The maxillary foramen is wide and elongated, located ventral to the lacrimal fossa, and connects with the infraorbital foramen via the infraorbital canal. The sphenopalatine and caudal palatine foramen are located medial to it and separated by a reduced transverse bone plate (Figure 4).
The orbital hiatus is wide, shallow, and at its level open: the optic canal, the orbital fissure, and in the same opening round foramen with the rostral alar foramen. The last one communicates with the caudal alar foramen through a short alar canal (Figure 5).

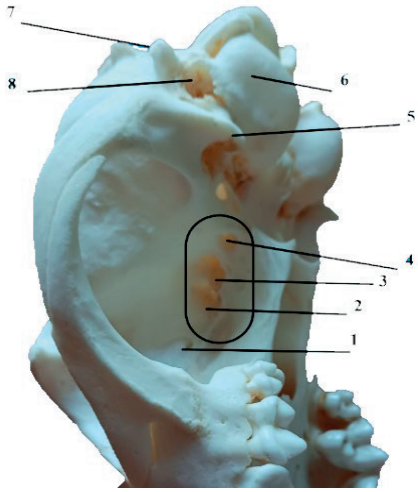

Figure 5. Lateral side of the jackal (*Canis aureus*) skull - original:
1. Ethmoidal foramen; Orbital hiatus: 2. Optical canal; 3. Orbital fissure; 4. Round/rostral alar foramen; 5. Retroarticular process; 6. Tympanic bulla; 7. Caudal process (occipital); 8. External acoustic meatus

Two highly developed, latero-medially convex, obliquely arranged tympanic bullae are on the ventral face in the aboral extremity. The hypoglossal canal is located between the occipital condyle and the tympanic bulla, slightly above the ventral condylar fossa. The jugular foramen is caudo-medially from the tympanic bulla (Figure 6).

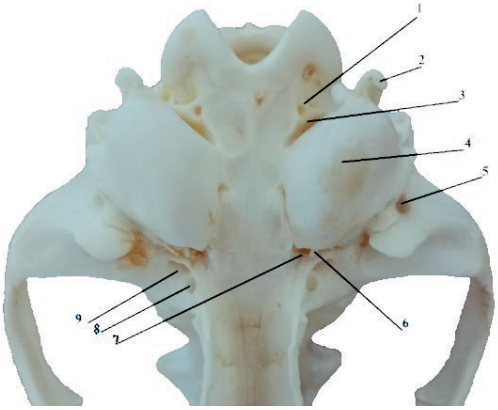

Figure 6. Ventral face of jackal (*Canis aureus*) skull – original:
1. Hypoglossal canal; 2. Jugular processes; 3. Jugular foramen; 4. Tympanic bulla; 5. Retroarticular foramen; 6. Styloid process; 7. Carotid foramen/Spinous foramen; 8. Caudal alar foramen; 9. Oval foramen

The paracondylar processes are attached to the caudal extremity of the tympanic bulla. They form a narrow and deep ventral condylar fossa with the occipital condyles.
The carotid foramen has a common wall with the spinous foramen at the rostral extremity of the tympanic bulla. Rostro-lateral to the carotid foramen is the oval foramen. The styloid process is significantly reduced (Figure 6).
Near the base of the tympanic bulla on the medial side, there is a small spheno-basioccipital tubercle. At the end of the long and straight pterygopalatine crest, a short pterygoid hamulus projects caudally.
The basioccipital body continues seamlessly with the basisphenoid body and features a medial crest that fades rostrally (Figure 7).

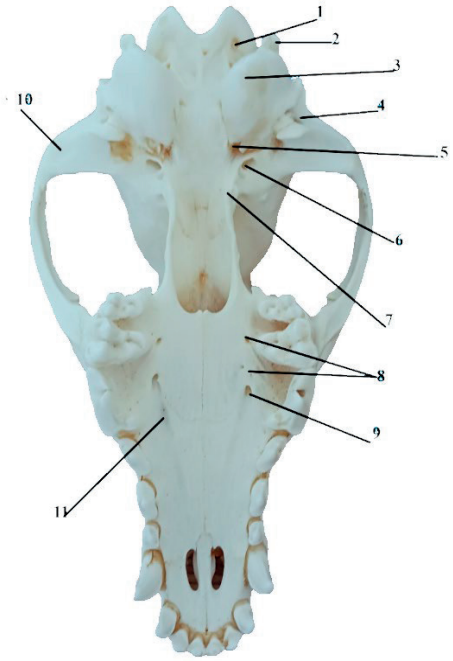

Figure 7. Ventral face of the jackal (*Canis aureus*) skull - original:
1. Ventral condylar fossa; 2. Jugular processes; 3. Tympanic bulla; 4. Retroarticular foramen; 5. Carotid foramen and spinous foramen; 6. Oval foramen; 7. Hamulus of the pterygoid bone; 8. Lesser and accessory palatine foramina; 9. Greater palatine foramen; 10. Zygomatic process of the temporal bone
11. Palatine groove

Located on the ventral side of the zygomatic process of the temporal bone, the mandibular fossa is a stretched-out glenoid cavity. Just beyond it lies a curved and well-developed retroarticular process that projects rostrally. Caudal from the base of the retroarticular process is an elongated dorso-caudal retroarticular foramen (Figure7).

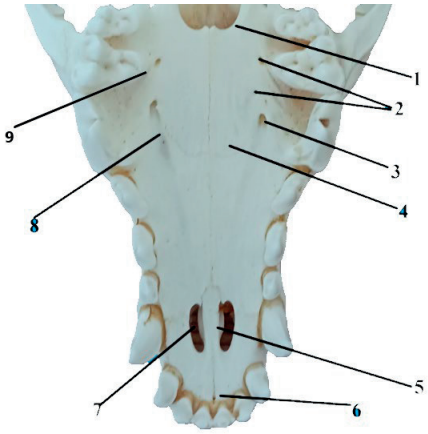

Figure 8. Hard palate in the jackal (*Canis aureus*) - original
1. The horizontal plate of the palatine; 2. Lesser and accessory palatine foramina; 3. Greater palatine foramen; 4. The palatine processes of the maxilla; 5. The palatine processes of the incisive; 6. Interincisive foramen; 7. Palatine fissure; 8. Palatine groove; 9. Lesser palatine foramen

On the hard palate, close to the alveolar process, the greater and the lesser palatine foramen are on each side. On the right side, 2-3 accessory palatine foramina appear caudally from the greater palatine foramen (Figure 8).
The palatine grooves are well highlighted and start from the greater palatine foramina, reaching the level of the palatine fissures, which are wide, oval, and arranged at the canine alveoli level.
The nuchal face has a triangular aspect, with developed nuchal crests on the sides; the maximum height is in the dorsal part. The external occipital protuberance is evident (Figure 9).
The external occipital crest is visible in the median plane, linking the external occipital protuberance and the foramen magnum (Figure 9).

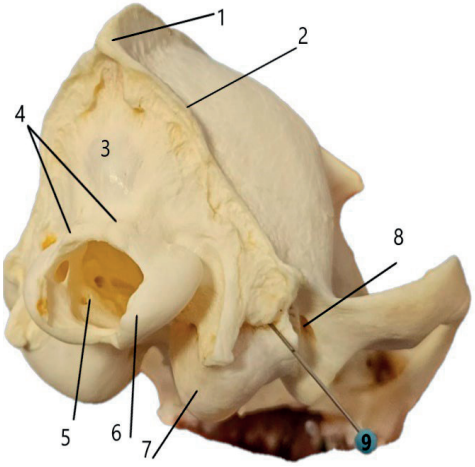

Figure 9. Nuchal face of jackal (*Canis aureus*) skull - original:
1. External occipital protuberance; 2. Nuchal crest; 3. External occipital crest; 4. Nuchal tubercles; 5. Foramen magnum; 6. Foramen magnum; 6. Occipital condyle; 7. Tympanic bulla; 8. Retroarticular foramen; 9. Stylomastoid foramen

The external acoustic meatus has the appearance of a wide and elliptical opening, and caudal to it, the stylomastoid foramen opens. The mastoid process of the temporal bone is reduced and has a tuberous appearance.

The foramen magnum is relatively oval and, on the dorsal margin, has two reduced nuchal tubercles. It is delimited on the sides by two condyles arranged slightly obliquely dorso-ventrally.

Above each condyle, there is a small dorsal condylar fossa. The paracondylar processes arranged approximately vertically are attached to the tympanic bulla, forming a narrow and deep ventral condylar fossa with the condyles.

The mandible is a paired bone in the jackal (*Canis aureus*). The part incisive of the mandibular body is short.

The diastema is reduced, but the dental alveolus for the canine is developed. The molar part of the mandibular body has a slightly convex ventral edge and ends with an apparent angular process, curved dorsally. There is a small fossa on the medial side of the angular process.

On the lateral face of the mandibular body, caudal to the mental foramen is an accessory mental foramen.

The lateral side of the mandibular ramus is a deep, triangular masseteric fossa that extends until the base of the coronoid process. On the medial side, the pterygoid fossa is shallow, and the mandibular foramen is arranged ventro-caudally (Figure 10).

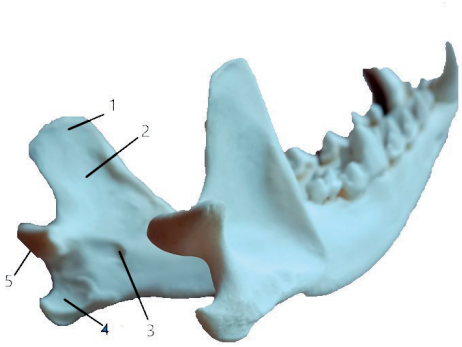

Figure 10. Jackal (*Canis aureus*) mandible - original:
1. Coronoid process; 2. Pterygoid fossa; 3. Mandibular foramen; 4. Angular process; 5. Condylar process

The coronoid process is well-developed and maintains a consistent width on its length; at its proximal end, the edge is rounded. The condylar process is short, with a convex surface, and the corono-condylar notch is reduced (Figure 11).

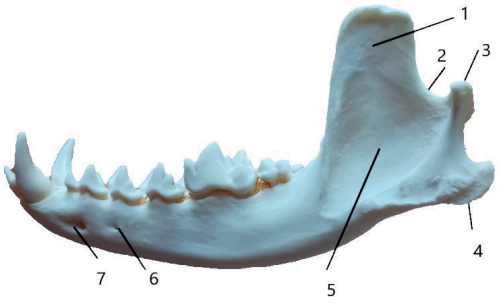

Figure 11. Jackal (*Canis aureus*) mandible - side view - original:
1. Coronoid process; 2. Corono-condylar notch; 3. The condylar process; 4. Angular process; 5. Masseteric fossa; 6. Accessory mental foramen; 7. Mental foramen

CONCLUSIONS

The jackal (*Canis aureus*) skull is dolichocephalic. The frontal bones show two well-defined nasal processes in the rostral extremity. The nasal bone is narrow and elongated and ends in the caudal extremity

through very sharp frontal processes. The nasal cavities communicate through a wide subvomerian space.

The orbit is incomplete. The supraorbital foramen is absent. There is a single lacrimal foramen and a single ethmoidal foramen.

On the occipital's squamous part, two nuchal tubercles are on the dorsal margin of the foramen magnum.

The tympanic bullae are highly developed, oblique and convex latero-medially. The jugular processes are arranged ventrally and are attached to it. At the rostral extremity of the tympanic bulla, the carotid and the spinous foramina have a single external opening. The styloid process is very reduced. The stylomastoid foramen is caudal to the external acoustic meatus.

The round foramen and the rostral alar foramen have a joint opening in the orbital hiatus. The short alar canal links the rostral and the caudal alar foramen.

Caudal to the retroarticular process is an elongated retroarticular foramen.

The maxillary tuberosity is reduced and has an apparent medio-aboral spine.

The coronoid process of the mandible is very developed, with approximately the same width along its entire length. There is a small fossa on the medial side of the angular process.

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CHARACTERIZATION OF THE METABOLIC RESPONSE OF LOHMANN BROWN LAYERS TO FEEDING DIETS WITH DIFFERENT PROTEIN LEVELS

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Abstract

40-week-old Lohman Brown layers were used in this study. Three diets with different levels of crude protein allowed the consumption of 15.38, 13.58 and 11.78 g/d/cap., respectively. The experimental feeding lasted four weeks. Laboratory determinations were performed at the end of the experimental period. Triglycerides, total cholesterol, GOT, GPT and γ-GT transaminases, HDL-cholesterol, protein, albumin, γ-globulins and uric acid were determined in the blood serum. Nitrogen intake, nitrogen excretion in manure, nitrogen retention in the body and in the egg were also determined. Thus, following four weeks of experimental feeding, serum transaminases and HDL-cholesterol remained within normal limits. Triclycerides and uric acid presented an increase of their serum values. As a consequence of the decrease in daily nitrogen intake, nitrogen excretion and nitrogen retention as total in the body decreased while nitrogen excretion as total in the egg remained unmodified.

Key words*: Lohmann Brown layers, diet protein level, metabolic response.*

INTRODUCTION

Housing and feeding are among the multitude of factors that influence the productivity of laying hens in general, the Lohmann Brown tetra-line laying hybrid in particular (Koreleski & Świątkiewicz, 2009; Wang et al., 2014). As early as 1972, Hurwitz and Bornstein started research on the protein requirements of laying birds establishing the basic benchmarks for calculating the protein requirements of laying hens. Later, Wang et al. (2014) searched the effects of protein source and nutrient density in the diets of broiler breeders on their subsequent growth, blood constituents, and carcass compositions. In Romania, Marmandiu et al. (2020) published a recent study on the influence of rearing system on laying in Lohmann Brown. Usturoi et al. (2022) studied the adaptability of the same Lohmannn Brown hybrid in different production systems. The two most important components of a diet in poultry are energy and protein. Nutritional requirements for crude protein and related amino acids are provided by NRC 1994.

Dietary protein is important in its level and origin. These two properties of dietary protein exert energetic influence but also influence digestibility, absorption, its use in intermediate metabolism, and excretion. The sources of protein used in the diet of birds are relatively numerous: from plant-based proteins such as soybean meal, cotton seed meal, alfalfa meal and sunflower meal, to animal-based protein such as fish-meal and poultry products. Numerous studies try to establish the optimal levels of protein in the diet, depending on the age and physiological state of the birds (Macelline et al., 2021). The metabolic influences of these protein sources are different. This is because some proteins are absorbed faster, others slower, some more and some less. The most preferred source of protein used in poultry diet formulation is the soybean. It contains up to 49% crude protein, depending on the removed hulls and the process of oil

extraction. The ultra-specialized commercial Lohmann Brown hybrid for egg production still presents many unknown particularities regarding nutritional physiology, especially protein requirements and intermediate protein metabolism. The present study aims to determine the effect of different dietary protein levels/sources on nitrogen balance in 40-week-old Lohmann Brown hens.

MATERIALS AND METHODS

Animals and experimental design

A total number of 120 40-week-old Lohmann brown hens devised in three equal groups were monitored in this experiment. The birds were raised in an industrial system benefiting of a program of feed restricted to 110 g/day, free access to water and a light program of 14:30 hours per day, from 5:30 to 20:00. The animals in the three groups were fed diets differing in the level of protein in the feed. The structure and chemical composition of the diets used in the experiment are presented in Table 1. Two diets were formulated: the first providing 15.38 g of protein at an average daily consumption of 110 g forage/capita and the second providing an amount of 11.78 g of protein at a consumption of 100 g of forage/capita. The combination of the two diets resulted in a third, the intermediate diet, which provided 13.58 g protein/capita.

The duration of the experimental monitoring period was from 40 to 44 weeks of age of the hens. The entire mass of faeces (mixture of faeces and urine) was collected daily, weighed and stored a -20°C until drying. Excreta samples were dried in a forced-air drying oven at 115°C for 72 h and finally ground for chemical analyses. Daily forage intake was restricted for 110 g/capita/day during the experimental period.

Analysis

Feed and excreta samples were analysed for dry matter (method 930.15; AOAC, 2005), total nitrogen and non-proteic nitrogen (after

Table 1. Ingredients and composition (%) of the diet to provide 13 g of protein, composition of the diet to provide 17 g of protein at 110 g and the composition of the intermediary diet to provide 15 g of protein at 110 g of daily feed intake each diet

Items	Control (diet before experimental feeding)	Diet 1: for 17 g of crude protein/day in 110 g of forage/day	Diet 2: Intermediary diet:15 g of crude protein/day in 110 g of forage/day	Diet 3: for 13 g of crude protein/day in 11 g of forage/day
Ingredients				
Corn meal	72.05	68.66	72.71	76.77
Soybean meal (in which, 48% crude protein)	11.80	13.43	11.87	10.32
Corn gluten meal	2.56	5.50	2.75	—
Soybean oil	2.00	2.00	2.00	2.00
Choline chloride	0.10	0.10	0.10	0.10
Limestone	8.00	8.00	8.00	8.00
Hen eggshell	0.42	0.40	0.43	0.45
Dicalcium phosphate	0.72	0.70	0.73	0.76
Salt	0.38	0.36	0.38	0.40
Premix of vitamins and minerals[1]	0.50	0.50	0.50	0.50
Nutrient contents (%, as calculated values)				
ME	2,925.1	2,867	2,928.5	2,990
Calcium	3.38	3.40	3.40	3.40
Nonphytate phosphorus	0.21	0.22	0.22	0.22
Protein	**13.2**	**15.38**	**13.58**	**11.78**
Nitrogen	2.13	2.44	2.15	1.86
Crude fiber	2.98	2.92	2.89	2.86
Crude fat	3.43	3.64	3.54	3.44
Ash	8.78	8.89	8.80	8.63

[1]Premix vitamino-mineral composition: vitamin A - 30,000 IU; vitamin D_3 - 4,500 mg; vitamin E -102 IU; vitamin K - 8 mg; vitamin B_1 - 8 mg; vitamin B_2 - 35 mg; vitamin B_6 - 22 mg; vitamin B_{12} - 0.10 mg; biotin - 0.20 mg; pantothenic acid - 86 mg; niacin, 70 mg; Fe - 150 mg; Zn- 30 mg and Se - 0.50 mg.

deproteinization by trichloroacetic acid) by Kjeldhal (AOAC, 199). Uric acid in excreta was determined by the enzymatic method of Marquardt (1983). Basically, the method consisted in the determination of the absorbance of a perchloric acid extract of the excreta measured at 285 nm versus an uric acid standard solution. Nitrogen balance including intake, excretion, and retention was calculated based on the method study proposed by Barzegar et al. (2019). Blood samples were taken from the axillary vein at the beginning and end of the experimental feeding period. Serum was obtained from the blood samples after coagulation, decantation and centrifugation at 1500 rpm. The serum samples thus obtained were used to determine serum biochemistry: total cholesterol, HDL-cholesterol, total triglycerides, proteins, total, albumin, globulins, GOT, GPT, γ-GT, inorganic nitrogen, and uric acid. Serum biochemistry determinations were performed according to the methods described by Manta et al. (1976).

Statistics

The differences between experimentally fed groups were statistically processed using a Microsoft Excel 2019 software. The differences between the experimental groups were analysed by Tukey test. The correlation of the dietary protein level evolution and the analysed items was tested by Pearson correlation coefficient. The differences between the values at the end of the experimental feeding period were also statistically compared with those at the beginning of the experimental feeding period. The differences between the groups were considered significant when the probability of the null hypothesis was below 5% ($P < 0.05$).

RESULTS AND DISCUSSIONS

Regarding the metabolic response of the Lohmann Brown adult layers to different levels of dietary protein, the general aspect of the determined parameters in the blood serum is that of their maintaining within the physiological limits. First of all, the maintenance of serum transaminases within physiological limits is noted ($P > 0.05$ for each experimental group), which reveals the maintenance of the normal functional state of the liver cell. These results are in agreement with the results reported by Heo et al. (2023) on pullets and Leghorn laying hens. A special situation is revealed by uric acid in the serum. The blood serum levels of uric acid show a decrease which is proportional with the level of dietary proteins. Uric acid is the main form of protein catabolism in birds. Its decrease with the levels of protein in diets points to a diminution of protein catabolism, with the possible decrease of the use of proteins for energy production.

Table 2. Some biochemical parameters as they are influenced by different protein levels in diet in Lohmann Brown layers following four weeks of experimental feeding

Item	Values in the week before experimental feeding (control)	Values, 4 weeks of experimental feeding			Significance as P, by Tukey test
		Diet 1	Diet 2 (intermediary)	Diet 3	
Total cholesterol, mg/dL	111±11	112±22	106±21	110± 32	0.033
Triglycerides, mg/dL	117.7±19	113.8±15	117.0*±21	214.3*±13	0.017
GOT, U/L	162±32	164±16	157±14	167±23	0.701
GPT, U/L	1.70±0.3	2.00±0.23	1.71±0.23	1.86±0.04	0.093
γ-GT, U/L	31.4±0.2	30.2±1.9	30.9±0.5	30.4±1.0	0.200
HDL cholesterol, mg/dL	78.7±2.2	83.6±3.6	77.8±10.2	72.7±2.4	0.054
Total protein, g/dL	4.04±0.5	4.10±1.0	4.06±0.8	3.97±0.9	0.111
Albumin, g/dL	2.42±0.33	2.49±0.32	2.41±0.28	2.40±0.23	0.212
Globulins, g/dL	1.66±0.32	1.61±0.09	1.60±0.05	1.52±0.32	0.432
Uric acid, mg/dL	3.14±0.43	3.26$^{\#}$±0.76	3.11±0.55	2.70$^{\#}$±0.54	0.003

Complete details for the protein content of each diet are given in Table 1

Values are given as mean ± standard error of mean

Each value represents the mean of a minimum seven samples ($n \leq 7$)

Values in the same line with the same superscript are statistically different ($P < 0.05$)

Abbreviations: GOT for glutamic oxaloacetic transaminase; GPT for glutamic pyruvic transaminase; γ–GT for gamma-glutamyl transaminase; HDL for high-density lipoprotein.

Pearson correlation coefficient was 0.30 for the evolution of uric acid level, which reveals a direct relationship of the serum uric acid and the diet protein levels. The maintaining of the HDL cholesterol levels in the blood serum reveals, together with the transaminases GOT and GPT, the maintaining of the proteo-synthetic capacity of the liver in these birds. These results are again in agreement with those reported by Heo et al. (2023) on growing pullets and on He Line brown layers. In their study on the effects of different dietary protein levels (from 18 to 12% crude protein) on performance, nitrogen excretion, and odour emission of growing pullets and laying hens, Heo et al. (2023) found that lowering of crude protein levels in the diets of the broilers did not modify significantly the main serum compounds. The idea of maintaining blood serum parameters within physiological limits under feeding conditions with different levels of dietary protein (15 and 16.5% crude protein) is also supported by Xin et al. (2022). These authors described a significant increase of triglyceride values, which is in agreement with our results on Lochmann Brown, meaning a particular shift of the energy metabolism.

Table 3 shows the results regarding the nitrogen balance of the three groups of Lohmann Brown hens fed with different levels of protein in the feed: 17, 15.5 and 13%. From the data presented in Table 3, it was found that the modification of the protein levels in the food led to the modification of the nitrogen intake as well as the daily nitrogen excretion, despite the hens consumed the same amount of forage and the three diets were iso-energetic. Daily nitrogen intake decreased by a percentage of 21% in hens fed with diet 2 and by 44.4% in hens fed with diet 3. Nitrogen excretion decreased by 35.5% in hens fed by diet 2 and 41.2% in hens fed by diet 3. Nitrogen retention as total in the body decreased by 1.8% in group fed on diet 2 and by 66% in group fed on diet 3 versus hen fed on diet 1. All the values were significantly different ($P < 0.05$) according to Tukey test. Surprisingly, the different levels of crude protein tested over a period of four weeks in adult Lohmann hens, at the top of laying, did not affect the nitrogen content of the whole egg, revealing the preferential distribution of the nitrogen by the organism in this direction, which is again in agreement with the data

Table 3. Nitrogen metabolism in Lohmann Brown layers following four weeks of experimental feeding with different protein level diets

Item	Values in the week before experimental feeding (control)	Values 4 weeks thereafter			Significance as P, by Tukey test
		Diet 1	Diet 2	Diet 3	
Daily feed intake (g/d)	110	110	100	110	ND
Daily manure (g/d, as fresh manure: faeces + urine)[a]	101±11	112.3±8	100±22	92.2±13.4	1.011
Daily nitrogen intake (g/cap./d)	3.65±0.13	3.48$^{\beta}$±0.5	2.78$^{\beta}$±0.21	1.97±0.32	0.000
Nitrogen excretion (g/cap./d)	1.20±0.0	1.89±0.18	1.22±0.05	1.11±0.11	0.505
Nitrogen retention as total in the body (g/cap./d)[b]	1.51±0.09	1.59$^{\$}$±0.22	1.56$^{\$}$±0.32	0.86±0.19	0.005
Nitrogen retention as integral egg (g/d)[c]	0.90±0.03	1.21±0.05	0.89±15	1.09±0.10	0.221
Nitrogen retention in the body (g/cap./d)[d]	0.65±0.11	0.70‡ ±0.21	0.66‡±0.5	0.31±0.04	0.001

Complete details for the protein content of each diet are given in Table 1

[a] Values in table are given as mean ± standard error of values from each week of experimental feeding

[b] Calculated as difference between nitrogen intake and nitrogen excretion

[c] Calculated according to Heo et al. (2023), from Miranda et al. (2015), based on the following equation: egg mass (g/hen/d) Ł N concentration in eggs (%), assumed N concentration in egg as 1.94%

[d] Calculated as difference between nitrogen retention as total and nitrogen retention in the egg.

Daily excreta resulted form daily collection of the faeces.

Values in the same line with the same superscript are statistically different ($P < 0.05$)

Nitrogen balance as intake and retentions was calculated according to Barzegar et al. (2019)

ND - not determined

reported by Heo et al. (2023), in He Line layers. Similar results were reported Alfonso-Avila et al. (2022) for broiler chickens, and Soares et al. (2019) for growing pullets.

According to our findings, excreta ash concentrations were decreased as the dietary crude protein concentrations decreased. According to Adeola et al. (2016), low crude protein-induced decrease of excreta ash can be attributed to a greater contribution of basal endogenous losses of minerals in low ash diet compared with that in high ash diet. Nitrogen in excreta comes from several sources: unabsorbed nitrogen from ingestion, nitrogen resulting from endogenous catabolism, eliminated via the kidneys, as well as microbial nitrogen (Soomro et al., 2018). The decrease of the excreta nitrogen level can be explained by the lower content of diet proteins, respectively, amino acids. Our results regarding nitrogen excretion in hens fed diets with different protein levels are also in agreement with those published earlier by Latshaw and Zhao (2011) who reported lower levels of nitrogen in the excreta of hens fed 17 g protein/ day (5.68% nitrogen of dry matter of manure) compared to hens fed on 13 g of protein per day (3.98% nitrogen of dry matter of manure). However, these authors did not specify nor the age, neither the breed of the chickens. Similarly, according to Heo et al. (2023), excreta output, nitrogen intake, and nitrogen excretion were linearly decreased (−0.12 Pearson correlation coefficient) as the crude protein levels decreased in the diets of the experimental layers, which was to be expected. It is reported that variations in lower limits of crude protein (16.5-18%) do not significantly change uric acid nitrogen in manure (Murakami et al., 2011).

CONCLUSIONS

The application of diets with different levels of crude protein in the feed in 40-week-old Lohmann Brown hens allowed the characterization of metabolic response of the hybrid. Thus, following four weeks of experimental feeding, serum transaminases and HDL-cholesterol remained within normal limits. The parameters that showed increased serum values were triglycerides and uric acid. As a consequence of the decrease in daily nitrogen intake, nitrogen excretion and nitrogen retention as total in the body decreased while nitrogen excretion as total in the egg remained unmodified.

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RESEARCH ON THE VALUES OF ELECTROCARDIOGRAPHIC PARAMETERS IN DOGS, ACCORDING TO BREED

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Abstract

In this study, we recorded and analysed electrocardiograms of different dog breeds. All the animals included in the study were healthy in terms of cardiac pathology. Upon analysing the obtained results, we found that Labrador Retrievers exhibited the highest P wave amplitude (0.40±0.05 mV). Regarding the R wave amplitude, high values were observed both in medium-sized breeds (Beagle – 1.70±0.16 mV) and large breeds (Rottweiler – 2.43±0.30 mV). As for the T wave amplitude, high values were observed in small breeds (Yorkshire Terrier – 0.24±0.14 mV) and medium-sized breeds (Cocker Spaniel – 0.24±0.14 mV), while large breeds (Labrador and Rottweiler) had the lowest values (0.21±0.14 mV). Regarding the duration of the main ECG components, it was observed that the P wave had the shortest duration (0.02±0.01 s) in Beagles and the longest duration (0.08±0.04 s) in Labrador Retrievers. The PR interval had the shortest duration (0.04±0.01 s) in Beagles and the longest duration (0.10±0.02 s) in Rottweilers. The QRS complex had the shortest duration (0.02±0.01 s) in Beagles. The QT interval had the shortest duration (0.16±0.01 s) in Yorkshire Terriers. The T wave had the same duration (0.04±0.01 s) for all examined breeds. Regarding heart rate, it was observed that larger dogs have a lower heart rate compared to smaller dogs.

Key words*: amplitude, duration, electrocardiogram, breed.*

INTRODUCTION

The electrocardiogram (ECG) represents the graphic recording or visualization of the cardiac action current on an oscilloscope and its use in clinical practice is important for obtaining information regarding the heart functions (Ghiță et al., 2016; Codreanu, 2018). The ECG is commonly used to investigate the heart rate, detect abnormalities within the excito-conduction system's integrity and diagnosing cardiac arrhythmias (Kumar et al., 2014; Bodh et al., 2016; Reetu et al., 2017; Ghiță et al., 2023). In small animal practice, electrocardiography can be used to detect possible atrial dilations (by modifying the P wave) as well as ventricular dilations (by modifying the R wave) (Ghiță et al., 2007; Neagu et al., 2015). Furthermore, by examining the electrocardiogram recorded using 6 leads (I, II, III, aVR, aVL, and aVF), the value of the mean electrical axis of the heart can be determined (Ghiță et al., 2007). In research, ECG is used to evaluate the effect of training or substances on cardiac activity (Leca et al., 2017). ECG recording is of particular practical importance, being used to calculate the duration of waves, segments, and intervals, as well as the amplitude of electrocardiographic waves (Ghiță et al., 2016). The main objective of our study was to determine the ECGs parameters of dogs with different weights and sizes: small-sized breeds (Yorkshire Terrier and Pomeranian), medium-sized breeds (Beagle and Cocker Spaniel) and large-sized breeds (Labrador and Rottweiler). The resulting values were then compared between each breed. With this purpose, we determined the amplitude (mV) of the P, R, and T electrocardiographic waves and the durations (s) of the P wave, PR interval, QRS complex, QT interval, and T wave. Another parameter of interest was the cardiac frequency (calculated electrocardiographically based on the RR interval). At the end of the study, we compared our results with those reported by other authors in the scientific literature.

MATERIALS AND METHODS

In our research, we used a portable veterinary electrocardiograph with 6 channels, enabling simultaneous recording of the electrocardiogram

in 3 bipolar leads and 3 unipolar leads. To establish contact with the animal's skin, we used rubbing alcohol (easy to apply, and no cleaning of the skin was required afterward), and metal alligator-type electrodes (providing good contact with the skin).

We recorded electrocardiograms from clinically healthy dogs presented at the clinic for routine procedures: deworming, vaccination, nail trimming, anal gland expression, cosmetic interventions, etc. We examined a total number of 46 dogs of various breeds, as follows: 15 small-breed dogs (8 Yorkshire Terriers and 7 Pomeranians), 15 medium-breed dogs (8 Beagles and 7 Cocker Spaniels), and 16 large-breed dogs (8 Labradors and 8 Rottweilers).

To record the ECGs, we used two systems: bipolar leads (I, II, and III) and unipolar leads (aVR, aVL, and aVF), with electrodes placed on the body surface as follows: the red electrode on the skin in the axillary area (on the right side), the yellow electrode on the skin in the axillary area (on the left side), the black electrode on the skin in the region of the groin fold (on the right side), and the green electrode on the skin in the region of the groin fold (on the left side). Recordings were conducted under electrical isolation conditions (to prevent loss or distortion of electrocardiographic signals) by placing the dogs in right lateral position on an examination table covered with a rubber mat.

We determined the following electrocardiographic parameters: amplitude of the P, R, and T waves, duration of the following components: P wave, PR interval, QRS complex, QT interval, T wave, and cardiac frequency (calculated based on the RR interval). The electrocardiographic parameters used were paper speed of 25 mm/sec and millivolt amplitude of 10 mm.

The obtained results are presented in tables and graphs accompanied by interpretations and explanations. For the statistical analysis of the results, we used the T Student test.

RESULTS AND DISCUSSIONS

In Table 1 and Figure 1, we present the results obtained, regarding the values of the electrocardiographic wave amplitudes in dogs, categorised by breed.

Table 1. Values of Electrocardiographic Wave Amplitudes (mV), according to breed (values are expressed as mean ± Standard Error)

Breed	P wave	R wave	T wave
Yorkshire terrier	0.25±0.04	1.25±0.26	0.24±0.014
Pomeranian	0.26±0.02	1.40±0.10	0.23±0.014
Beagle	0.23±0.03	1.70±0.16	0.23±0.014
Cocker spaniel	0.21±0.10	1.55±0.25	0.24±0.014
Labrador Retriever	0.40±0.05	2.30±0.20	0.21±0.014
Rottweiler	0.39±0.04	2.43±0.30	0.21±0.014

Analysing the data presented in Table 1 and Figure 1, it is observed that the large breeds have the largest P-wave amplitude, which means that atrial depolarization generates a stronger current than in the case of medium and small breeds. The observed differences are not statistically significant.

Regarding the amplitude of the R wave (Table 1 and Figure 2), high values are observed in both medium breeds (Beagle) and large breeds (Labrador and Rottweiler). The highest value (2.43 mV) is observed in the Rottweiler breed, and the value obtained by us falls within physiological limits (the maximum value being 2.5 mV) (Gonul et al., 2002; Cotor et al., 2014).

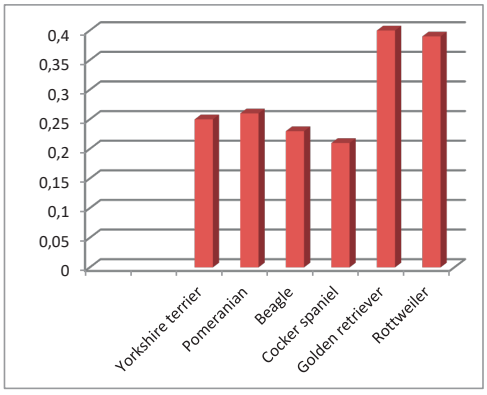

Figure 1. Mean values of the P wave amplitudes (mV), according to breed

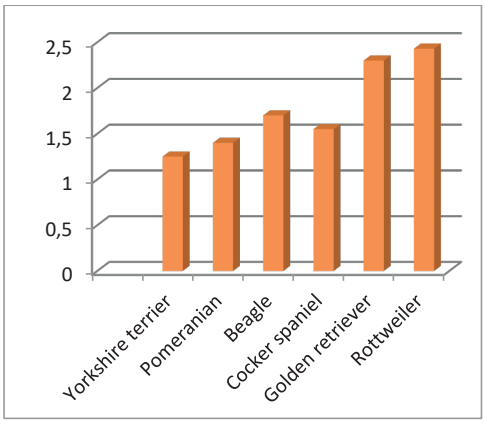

Figure 2. Mean values of the R wave amplitudes (mV), according to breed

Regarding the amplitude of the T wave (Table 1 and Figure 3), high values are observed in small breeds (Yorkshire Terrier) and medium breeds (Cocker Spaniel), while large breeds have the lowest values. For all studied breeds, the values fall within physiological ranges, with the T wave amplitude always being less than 1/4 of the R wave amplitude (Su et al., 2001; Mukherjee et al., 2015).

It should be emphasised that large breeds have the highest amplitude of the P and R waves and the lowest amplitude of the T wave.

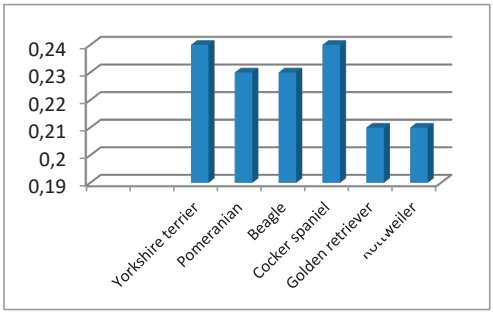

Figure 3. Mean values of the T wave amplitudes (mV), according to breed

In Table 2 and Figure 4, we present the results obtained, regarding the durations of the main electrocardiographic components in dogs, categorised by breed.

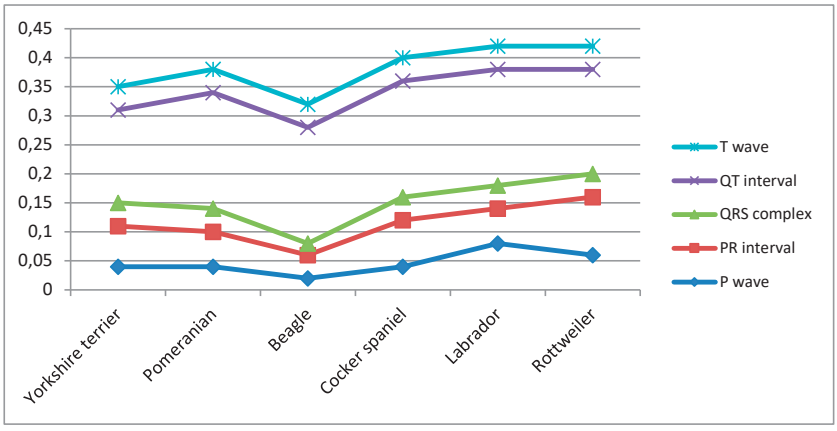

Figure 4. Mean values of electrocardiographic components, by breed

Analysing the details presented in Table 2 and Figure 4, the following distinctions can be made:

- The duration of the P wave ranges from 0.02 ± 0.01s (Beagle breed) to 0.060 ± 0.02s (Rottweiler breed) and 0.08 ± 0.04s (Labrador breed). In the case of these breeds, the differences are significant ($p < 0.05$) compared to the values recorded for other studied breeds.
- The duration of the PR interval is the shortest in the Beagle breed (0.04 ± 0.01s) and the longest in the Rottweiler breed (0.10 ± 0.02s). It is noteworthy that in the case of the Rottweiler breed, the differences observed by us are statistically significant ($p < 0.05$) compared to the values obtained for other breeds.
- The duration of the QRS complex was the same for all examined breeds (0.04 ± 0.01s), except for the Beagle breed where the obtained value was 0.02 ± 0.01, and this difference was not statistically significant ($p > 0.05$).
- The duration of the QT interval ranged from 0.16 ± 0.01s in Yorkshire Terrier to 0.18 ± 0.01s

for the Rottweiler, and 0.2 ± 0.01s in the other studied breeds. Statistical analysis did not reveal significant differences for any studied breed ($p > 0.05$).

- The duration of the T wave was 0.04 ± 0.01s for all studied breeds.

Table 2. Values of Electrocardiographic Component Durations (s) according to breed (values are expressed as mean ± Standard Error)

Breed	P wave	PR interval	QRS complex	QT interval	T wave
Yorkshire terrier	0.04±0.01	0.07±0.01	0.04±0.01	0.16±0.01	0.04±0.01
Pomeranian	0.04±0.01	0.06±0.01	0.04±0.01	0.2±0.01	0.04±0.01
Beagle	0.02±0.01*	0.04±0.01	0.02±0.01	0.2±0.01	0.04±0.01
Cocker spaniel	0.04±0.01	0.08±0.01	0.04±0.01	0.2±0.01	0.04±0.01
Labrador	0.08±0.04*	0.06±0.02	0.04±0.01	0.2±0.01	0.04±0.01
Rottweiler	0.06±0.02*	0.10±0.02*	0.04±0.01	0.18±0.01	0.04±0.01

In Table 3 and Figure 5, we present the results obtained by us regarding the heart rate duration in dogs, categorised by breed (calculated electrocardiographically based on the R-R interval).

Table 3. Heart rate values (bpm) according to breed (values are expressed as mean ± Standard Error)

Breed	HR
Yorshire terrier	135.00±10.2
Pomeranian	145.00±5.00
Beagle	106.25±3.75
Cocker spaniel	140.50±15.5
Labrador	115.00±12.3
Rottweiler	125.00±10.2

It is worth mentioning that for all analyzed breeds, the results fall within the values mentioned in the specialized literature (Sato et al., 2000; Hanton et al., 2006; Gugjoo et al., 2014). One possible explanation for this finding could be the presence of a more intense metabolism in small breeds, leading to a greater need for oxygen at the tissue level, which can also result in a more intense cardiac activity.

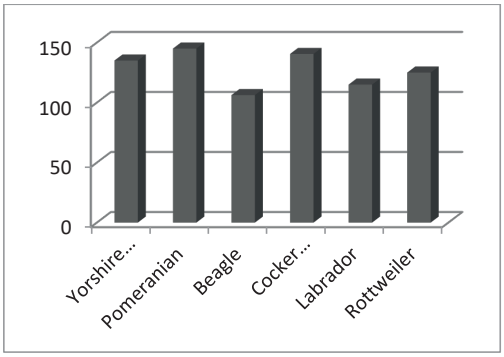

Figure 5. Mean heart rate values (bpm) in the studied dogs, according to breed

Table 4. Electrocardiographic wave amplitudes (mV) recorded in dogs (according to other authors)

Author	P wave	R wave	T wave
Tilley L.P. et al.	<0.40	<2.5 small breeds <3 large breeds	<¼ of R wave
Martin M.	<0.40	<2 small breeds <2.5 large breeds	<¼ of R wave
Kittleson M.D. et al.	<0.40	<3	-
Johathan A. et al.	0.40	2.5-3.0	<¼ of R wave
Mike W.S. şi col.	0.40	<2 small breeds <2.5 large breeds	<¼ of R wave
Philip R. Fox	0.40	<2.5 small breeds <3 large breeds	<¼ of R wave
Personal research	<0.26 small breeds <0.23 medium breeds <0.40 large breeds	<1.40 small breeds <1.70 medium breeds <2.43 large breeds	<0.24 small breeds <0.24 medium breeds <0.21 large breeds

Comparing our data (Table 1) with those reported in the specialised literature (Table 4), it is observed that our data fall within the standard limits mentioned. Regarding the durations of certain electrocardiographic components, we compared our values with those obtained by

various authors. In the present study, we determined the durations of the P wave (representing atrial depolarisation), PR interval (representing atrial electrical systole), QRS complex (representing ventricular depolarisation), QT interval (representing ventricular electrical systole), and T wave (representing ventricular repolarisation).

Table 5. Duration of electrocardiographic components (seconds) recorded in dogs (according to other authors)

Author	P wave	R wave	T wave
Tilley L.P. et al.	<0.40	<2.5 small breeds <3 large breeds	<¼ of R wave
Martin M.	<0.40	<2 small breeds <2.5 large breeds	<¼ of R wave
Kittleson M.D. et al.	<0.40	<3	-
Johathan A. et al.	0.40	2.5-3.0	<¼ of R wave
Mike W.S. et al.	0.40	<2 small breeds <2.5 large breeds	<¼ of R wave
Philip R. Fox	0.40	<2.5 small breeds <3 large breeds	<¼ of R wave
Personal research	<0.26 small breeds <0.23 medium breeds <0.40 large breeds	<1.40 small breeds <1.70 medium breeds <2.43 large breeds	<0.24 small breeds <0.24 medium breeds <0.21 large breeds

Comparing the results obtained by us (Table 2) with those obtained by other authors (Table 5), we observe that there are no major differences between our results and those encountered in specialized literature.

CONCLUSIONS

Regarding the amplitude of the electrocardiographic waves, we observed that the P wave has the highest value in large breeds (Labrador), the R wave has high values both in medium breeds (Beagle) and in large breeds (Rottweiler), and the T wave has high values in small breeds (Yorkshire Terrier) and medium breeds (Cocker Spaniel), with large breeds (Labrador and Rottweiler) having the lowest values.

Regarding the duration of ECG components, we observed that the duration of the P wave is the shortest in the Beagle breed and the longest in the Labrador breed. The duration of the PR interval is the shortest in the Beagle breed and the longest in the Rottweiler breed. The duration of the QRS complex was the same for all examined breeds except for the Beagle breed, where it was shorter. The duration of the QT interval was the shortest in Yorkshire Terriers. The duration of the T wave was identical for all studied breeds.

Regarding heart rate, we observed that large-sized dogs have a lower heart rate than small-sized dogs.

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EFFECTS OF PRE-LAY DIFFERENT PROTEIN AND ENERGY DIETARY LEVELS ON SUBSEQUENT PERFORMANCES IN LOHMANN BROWN

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Abstract

The aim of this paper was to find out the effects of diets with different levels of metabolizable energy (ME) and crude protein (CP) administered during the pre-lay period on the subsequent productive performances of the Lohmann Brown hybrid. A low ME diet (2,690 kcal/kg) and a high ME diet (2,820 kcal/kg were prepared, each one with two variants of CP levels: low (15%) and high (17%). These diets were applied from 15 to 22 weeks of age. From 23 weeks of age, all birds were fed a diet containing 2,700 kcal/kg and 15.6% protein. Egg rate was significantly higher in the high-protein-high-ME fed group. In the same group, the intensity of the yolk colour decreased and the level of total protein concentration in the albumen was higher. Other researched productive parameters (feed intake, egg mass, feed conversion rate) and egg properties (eggshell colour, eggshell breaking strength, Haugh units) were found not significantly modified by the experimental pre-lay feeding.

Key words*: Lohmann hybrid, metabolic energy, production performance, egg features.*

INTRODUCTION

The Lohmann Brown hen originates from Germany. Since then, Lohmann Brown hen have become the best hybrid layer due to its large, brown-shelled eggs (Lohmann Management Guide, 2024). The Lohmann Brown hybrid is a cross-breed of the White Rock and Rhode Island Red hen breeds or strains. This Lohmann Brown has been widely used in the egg industry from Germany to other parts of the world. It is also one of the earliest hybrids developed and is still in demand today due to its excellent productivity, egg quality and adaptability (Lohmann Management Guide, 2024). This Lohmann hybrid is the most widespread laying hens in the worldwide. Among the productive performances of this egg-laying hybrid, we specify: a production of 337.5 eggs in a laying period from 19 to 80 weeks of age, egg breaking resistance of over 35 newton, an egg average weight of 65 g, a feed conversion index of 2.1-2.2 kg feed/kg egg mass, and a daily *ad libitum* consumption of 110-120 g feed (Văcaru Opriș et al., 2002). Sensibility to some parasitic diseases and tuberculosis, predisposition to moulting, nutritional requirements, sensibility to housing conditions and a poor carcass quality are some of the hybrid's problems. As a product of an intense process of selection and amelioration, itself still subject to an intensive selection and amelioration pressure, the hybrid attracted the attention not only of selectors and breeders, but also of other categories of researchers, including those of nutrition physiology (Usturoi et al., 2006; Usturoi et al., 2022). The work of specialists in the nutrition physiology of this hybrid is focused on creating diets that respond as precisely as possible to its nutritional requirements. Digestive absorption and feed conversion capacities (Alagawany et al., 2016), response to different dietary protein sources (Wang et al., 2014), different protein

levels and different energy levels (Kim, 2014; Xin et al., 2022) of the diet are just a few examples of the lines of investigation. The purpose of this work is to investigate the effects of different dietary protein and energy levels during the pre-lay period on subsequent laying performances and egg features in Lohmann layers. The data will be used to establish diets that respond as accurately as possible to the requirements of the species during the egg-laying period.

MATERIALS AND METHODS

Experimental design

The experiment was carried out on 360 Lohman brown hens. The birds were grouped into four equal groups of 90 cap. each. The four groups of 90 birds each were housed in four 5.6/4 m cages, totalling 22.4 m^2 each, achieving a density of 4 cap./m^2. Four experimental diets were prepared: two low metabolic energy diets and two high metabolic energy diets. Each of the two types of diets with different levels of metabolic energy had in their composition different levels of protein (low and high, Table 1). The four experimental diets were administered *ad libitum* from 15 to 22 weeks of age. From 23 weeks of age all individuals in the four groups were fed the same new diet, formulated according to National Research Center (NRC), Nutrient recommandations for poultry (1994), with a content of 2700 kcal/kg feed and 15.6 g crude protein/100 kg feed (Table 1). The room temperature was maintained at 22 ± 2°C throughout the study. Light period was 8 hour/day (7:00-15:00) at 15 weeks of age, then increased gradually by 1 hour/week till 14 hours/day (5:00-19:00) until 22 weeks of age of the birds.

Egg production, daily feed consumption and body weight were monitored through the experiment.

Table 1. Metabolic energy, protein, ingredients and nutrient composition of the diets formulated for experimental groups for pre-laying (15-22 weeks) and laying (23-50 weeks) periods

Energy	Experimental pre-laying feeding period (15-22 weeks)				Laying feeding period (23-50 weeks)
	Low level ME		High level ME		
Protein	Low level CP	High level CP	Low level CP	High level CP	
Ingredients					
Corn	63.1	61.0	61.1	60.2	63.9
Soybean meal	17.1	22.2	16.9	22.4	22.7
Oil	-	-	2.1	1.6	1
Bran	15	12	16	11	-
Limestone	1.74	1.74	1.74	1.74	9.5
Choline chloride	0.08	0.08	0.08	0.08	0.08
Mono-dicalcium phosphate	0.65	0.65	0.65	0.65	0.78
Salt	0.31	0.31	0.31	0.31	0.31
Medical Stone	1.95	1.95	1.95	1.95	0.97
Lysine	0.1	0.1	0.1	0.1	0.12
Methionine	0.07	0.07	0.07	0.07	0.12
Vitamin and mineral premix[1]	0.25	0.25	0.25	0.25	-
Vitamin and mineral premix[2]	-	-	-	-	0.35
Nutrient composition					
Crude protein[3]	**15.11**	**16.92**	**14.77**	**16.78**	**15.60**
ME, Kcal/kg[3]	**2,690**	**2,690**	**2,820**	**2,820**	**2,700**
Calcium, %	0.85	0.85	0.85	0.85	3.55
Available phosphorus, %	0.56	0.56	0.56	0.56	0.49
Lysine, %	0.79	0.79	0.79	0.79	0.78
Methionine, %	0.30	0.30	0.30	0.30	0.40

[1]Provided per kg of premix: vitamin A, 160,000 IU; vitamin D, 66,000 IU; vitamin E, 550 mg; vitamin K, 40 mg; vitamin B_1, 60 mg; vitamin B_2, 130 mg; vitamin B_6, 50 mg; pantothenate, 220 mg; nicotinamide, 500 mg; folic acid, 10 mg; biotin, 6 mg, Cu, 0.30 g; Fe, 1 g; Mn, 2.50 g; Zn, 2.00 g; I, 13 mg; Se, 10 mg

[2]Provided per kg of premix: vitamin A, 220,000 IU; vitamin D, 72,000 IU; vitamin E, 550 mg; vitamin K, 80 mg; vitamin B_1, 50 mg; vitamin B_2, 160 mg; vitamin B_6, 70 mg; vitamin B_1, 1 mg; pantothenate, 220 mg; nicotinamide, 700 mg; folic acid, 30 mg; biotin, 6 mg, Cu, 350 g; Fe, 1.5 g; Mn, 3.0 g; Zn, 2.5 g; I, 20 mg; Se, 20 mg

[3]Calculated values

Egg quality

Egg weight was determined using an ORMA model analytical balance. Shell thickness was measured in the equatorial zone of the egg using an EGG Shell Thickness Gauge tester, Ro.Ma.snc Italia. The breaking strength of the eggs was determined with the Egg Force Reader: the device measures the minimum force required to break the egg shell, expressing the results in mg/cm^2. Determination of egg white consistency was performed using an EGG Analyser and was expressed in Haugh units. The colour intensity of the eggshell was analysed using a Model Novo-Shade Duo Colour Reflectometer, the comparison being assimilated to the colour shade assessment system provided by the manufacturer of the analysed hybrid. Yolk colour was determined using a combination of two methods: a method that uses the "Roche Yolk Colour Fan" colour palette with values from 1 to 15. Albumen protein was determined by Kjeldahl method (AOAC, 1995).

Statistics

The data were statistically processed by determining the mean and the standard error of the mean, using a dedicated software. The differences between the groups were analysed based on the Student's t test, being considered significant at $P < 0.05$.

RESULTS AND DISCUSSIONS

The analysis of the productive parameters presented in Table 2 shows the following particularities.

Table 2. Effect of dietary metabolizable energy and crude protein levels during the pre-lay period (15-22 weeks of age) on the productive performance of Lohmann brown from 23 to 50 weeks of age (mean values)

Item	Low protein diets		High protein diets		SEM	P value
	Low ME diet	High ME diet	Low ME diet	High ME diet		
Feed intake (g/d)[1]	118.1	121.1	118.8	117.3		
Body weight (g)[2]	1,921±32	1,956±44	1,911±33	1,964±51	63	0.611
Laying rate (%)[1]	82.6±2.2[#]	83.9±0.8	83.3±3.0	86.3±3.0[#]	5.33	0.011
Egg mass (g/d/cap.)[3]	55.3±5.2	52.5±3.2	52.1±5.3	54.3±4.8	6.33	0.329
Egg weight (g)[1]	62.7±6.5	62.6±5.3	62.6±4.3	63.0±4.0	4.79	0.049
Feed conversion rate (kg food / kg egg)[1]	2.28±0.34	2.29±0.66	2.28±0.21	2.16±0.11	0.43	0.096

[1]Calculated as mean from 23 to 50 weeks of laying
[2]Mean values at the end of experimental monitoring (50-wk-old hens)
[3]Calculated by multiplying the egg production by the egg weigh expressed as mean of weekly values
Values with the same superscript are statistically different
SEM = standard error of mean
Values as mean± standard error of mean

The average feed consumption during the period of 23 to 50 weeks of age of the birds did not show significant differences, being between 117.3 and 121 g/day/cap. The body weight at week 50 of age of the birds was higher in hens fed high ME diets but the difference remained no significant ($P>0.05$). The average egg laying rate during the period 23-50 weeks of age was the only productive parameter with significant differences between the groups ($P < 0.05$), the higher values being of the group fed high protein high ME diet. The same group presented the lowest feed conversion rate: 2.16 kg food/kg egg mass. Average egg weight and average egg mass were not significantly influenced during the monitored period (23-50 weeks), after feeding the four experimental diets. From the analysis of the data presented in Figure 1 it can be seen that the high-protein-high-ME diet fed group was the first to reach the laying peck and the egg laying values were above those of the other three groups during the following monitoring period, from 23 to 50 weeks of age. In the same period, the egg laying curve of the low-protein-low-ME group was placed below those of the other groups during the whole period. The graphic evolution shows that in the last three weeks of monitoring, the curves of the four batches are approaching, revealing the disappearance of the differences between the groups.

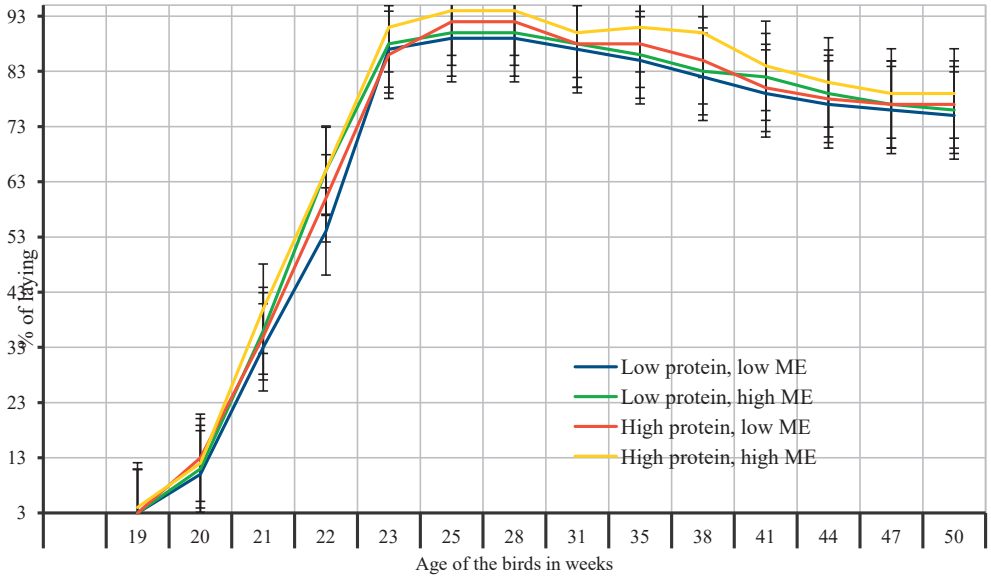

Figure 1. Evolution of laying (as % /housed hens) during experimental feeding and thereafter (23-50 weeks of age)

The results obtained by us on the Lohmann brown hybrid are partially in agreement with the data published by other authors (Xin et al., 2022) in their study on aged He Line brown hens. These authors found that diets with 15 or 16% crude protein and energy levels of 2.700 or 2.800 kcal did not significantly influence the egg production, egg weight, egg mass, and feed intake. Other researchers (Summers et al., 1994**)** tested diets with crude protein levels of 20, 17, 14, and 11% and found that decreased egg weight proportionally to decreasing protein content of the diet in White Leghorn hens. According to our results, we noted a decrease in the use of feed protein as Lohmann brown birds get older, which is in agreement with the data published by other authors (Schwartz & Allen, 1981) in Japanese Quail females, the aspect being a subject of further investigation.

Our results regarding the increase in laying rate and decrease in feed consumption rate in hens that were fed diets enriched in protein are also confirmed by other authros (Reid, 1976): this author reports a feed consumption rate of 1.89 kg/dozen eggs on a diet with 19% protein, values that increases progressively with the decrease in the percentage of crude protein in the diet. On a range of values from 19 to 13% crude protein, other authors (Heo et al., 2023) studied the effects of feed protein levels on egg properties finding no significant effects on egg physical properties (breaking strength, shell colour) but a significant effect on egg weight. The same authors also found no significant effects on daily feed consumption.

In our study, the analysis of egg properties (Table 3) reveals some significant influences, which maintained until the end of experimental feeding. A higher protein concentration of albumen ($P < 0.05$) was found in hens fed on protein enriched diets. Also, the shell thickness and egg shape index (not the breaking strength of the eggs) are significantly higher ($P < 0.05$) in the groups fed on protein-enriched diets. Significant influences of the level of metabolizable energy of the diets were not identified at this age of the birds.

In their study regarding the effects of different levels of crude protein and metabolizable energy in the diet in the pre-lay period on egg characteristics, some authors (Xin et al., 2023) found that at the age of 72 weeks of the birds, only the egg shape index, eggshell thickness and yolk colour still retain significant influences.

Table 3. Effect of dietary metabolizable energy and crude protein levels during the pre-lay period (15-22 weeks of age) on the egg features of Lohmann brown hen (mean values of 30 eggs from five working days in the 50th week of life)

\Item	Low protein diets			High protein diets			SEM	P value
	Low ME diet	High ME diet	Mean	Low ME diet	High ME diet	Mean		
Egg shape index	1.36	1.39	1.38[a]	1.34	1.37	1.36[a]	0.23	0.011
Eggshell breaking strength (kg/cm^2)	3.32	3.43		3.44	3.21		0.54	0.321
Eggshell thickness (μm)	330	330	330[b]	355	360	354[b]	54	0.110
Eggshell colour (absorbance)*	55	58		60	60		33	0.202
Yolk colour intensity**	6.6	6.8	6.7[c]	6.3	6.0	6.22[c]	1.32	0.032
Haugh unit (egg freshness)	78.2	77.7		81.0	80.3		18.5	0.065
Albumen protein conc. (mg/mL)	124.3	131.0	127.6[d]	126.4	133.0	129.7[d]	32.3	0.087

*Values expressed by the degree of light absorption from 0 to 100
**Mean values of a minimum three readings on the colour scale from 1 to 15
Values with the same superscript differ significantly

The other investigated egg properties in the experiment (eggshell breaking strength, yolk colour and Haugh units) no longer retained significant influences from energy and crude protein differentiated diets during the pre-lay period. The analysis of the graphic evolution of the total protein content of the albumen shows that these differences between the groups appeared as early as the age of 23 weeks of the birds, the differences reducing with increasing age (Figure 2). On the other hand, some researchers (Heo et al., 2023) in experiments on 13-week-old He Line brown found the increase in the intensity of the colour of the yolk proportional to the decrease in the crude protein content of the diet. The explanation given by Moros et al. (2022) and Galobart et al. (2004) was that this effect was secondary to the increase in corn gluten concentration of low crude protein diets. It is well known that corn gluten meal is rich in natural pigments (e.g., xanthophylls) for egg yolk colouration.

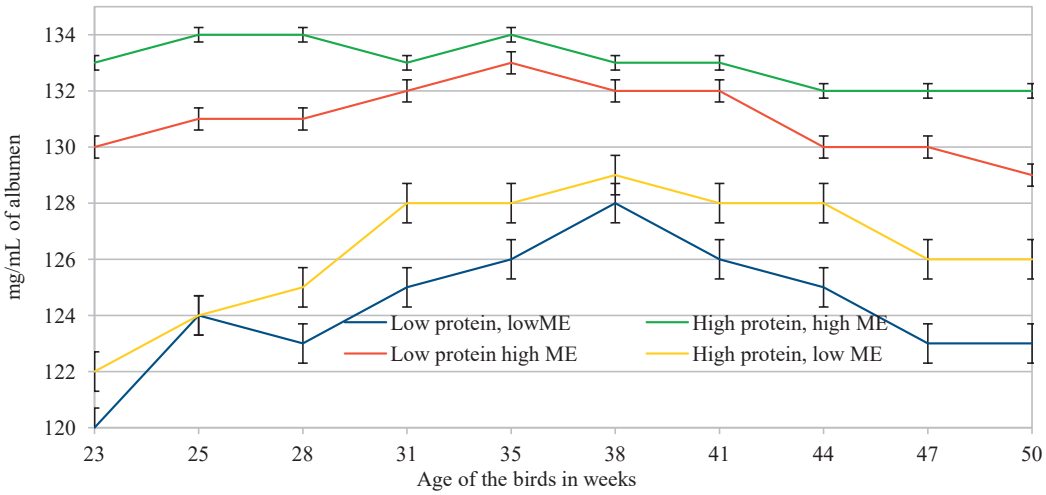

Figure 2. Evolution of the total albumen protein concentration in Lohmann brown hens fed on different protein and EM level diets in prelay period (15-22 weeks)

CONCLUSIONS

Feeding Lohmann brown chickens on diets containing different protein and ME levels in the pre-lay period (15-22 weeks) allowed the characterization of the effects on the productive performance and egg features during the subsequent laying period. Egg rate was significantly higher in the high protein high ME group. In the same group, the intensity of the yolk colour decreased and the level of total protein concentration in the albumen was higher. Other studied productive parameters (feed intake, egg mass, egg consumption rate) and egg properties (eggshell colour, eggshell breaking strength, Hough units) were not significantly modified by the experimental pre-lay feeding.

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ACUTE CONGESTIVE HEART FAILURE SECONDARY TO TRANSIENT MYOCARDIAL THICKENING IN A CAT: CASE REPORT

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Abstract

A one-year-old Scottish fold male cat was referred to the Cardiology department, presenting signs of congestive heart failure one week after anesthesia. Echocardiography revealed an enlarged left atrium with the left ventricular wall thickening and confluent "B lines". Cardiac biomarker troponin I was markedly elevated. The cat was discharged home after an intensive hospitalization treatment that led to progressive clinical improvement. It was reevaluated after two months, and a physical examination, echocardiographic measurements, and cardiac troponin were normalized. The diagnosis of transient myocardial thickening was considered based on the presence of a trigger possibly related to myocardial injury and the patient's history, cardiological, and laboratory data. This condition is relatively uncommon and closely resembles hypertrophic cardiomyopathy but involves reversions of the cardiac modifications. It usually appears in young cats with previous events. Transient myocardial thickening has a better prognosis in cats in contrast with hypertrophic cardiomyopathy. This case report describes a case of transient myocardial thickness with congestive heart failure, which returns to a case of normal cardiac features.

Key words*: feline, myocardial thickening, troponin I, echocardiography.*

INTRODUCTION

The idiom 'transient myocardial thickening' (TMT) was submitted in veterinary medicine in 2018 to describe a clinical pathology that resembles hypertrophic cardiomyopathy (HCM) and usually affects young cats (Matos et al., 2018).
Preceding articles in humans have shown that transient myocardial thickening was associated with hypertrophic cardiomyopathy secondary to acute myocarditis, atypical cases of stress-induced cardiomyopathy (Takotsubo), and storage diseases (Amyloidosis) (Kudo et al., 2011; Madias et al., 2013; Hwang et al., 2014; Elliot et al., 2014; Madias et al., 2016).
TMT was described by the increased diameter of the left ventricular walls, often with concurrent left atrial (LA) dysfunction and enlargement triggering congestive heart failure (CHF) and occasionally thromboembolism (ATE) in correlation with the increased concentration of cardiac troponin I (cTnI) in the bloodstream, suggesting myocardial injury (Matos et al., 2018; Langhorn et al., 2014; Vollmar et al., 2024).
This condition has a reversible character, improved cardiac function instead of progressive degradation of cardiac parameters associated with HCM, and a better prognosis. However, there is typically no clinical or echocardiographic evidence to differentiate the two disease processes at presentation. Cats with TMT usually had antecedent stressful events such as general anesthesia (Glaus et al., 2010).
This case report aims to describe the historical, clinical, diagnostic, therapeutic, and clinical evolution of a patient with transient myocardial thickening with secondary congestive heart failure, focusing on echocardiographic characteristics.

MATERIALS AND METHODS

Toby, an 8-month-old male British Shorthair cat, presented with dyspnea, hind leg instability, and intolerance to minimal effort, requiring emergency consultation. The patient had undergone anesthesia for orchidectomy and correction of entropion a week prior. During the physical examination, Toby weighed 5 kilograms, with a rectal body temperature of 39.0°C, capillary refilling time of 2 seconds, cardiac frequency of 140 beats per minute, Doppler front right limb systolic blood pressure

90 mmHg (cuff 2.0), and respiratory rate of was 60 respiration per minute with abdominal and restrictive breathing pattern. Pulmonary auscultation revealed severe, disseminated crackles on both hemithorax. Parasternal cardiac auscultation detected a high-grade systolic murmur accentuated on the left side, and the femoral pulse was strong and symmetrical. The cat was vaccinated and dewormed, feline immunedeficiency antibody /feline leukemia antigen virus (Idexx SNAP Combo) was assessed negatively. The attending physician performed blood sample tests, including a count of blood cells and quantitative pro-brain natriuretic peptide (NT- pro-BNP). The CBC showed mild neutropenia 2.92 x 10^9/L (normal range 3.12-12.58 x 10^9/L), mild monocytosis 0.05 x 10^9/L (reference range 0.07-1.36 x 10^9/L), and mild erythrocytosis 10.39 x 10^12/L (normal range 4.6-10.2 x 10^9/L). The NT-pro BNP is a hormone that belongs to the natriuretic peptide families. Cardiomyocytes secrete it in response to an abnormal extent, pressure, or volume overload (Connolly et al., 2018). The level of NT-pro BNP was mildly elevated at 63.6 pmol/L (normal values less than 50 pmol/L).
An additional blood test was performed after the clinical examination to determine if there was any ischemic myocardial injury. The results indicated that the cardiac troponin I level was significantly high, exceeding 20 ng/ml. This value is higher than the normal range, less than 0.06 ng/ml, indicating recent myocardial injury. The 6-lead electrocardiogram showed an average heart rate (140 bpm), sinus rhythm with standard QRS configuration, and axis with infrequent premature ventricular contractions (Figure 1A).

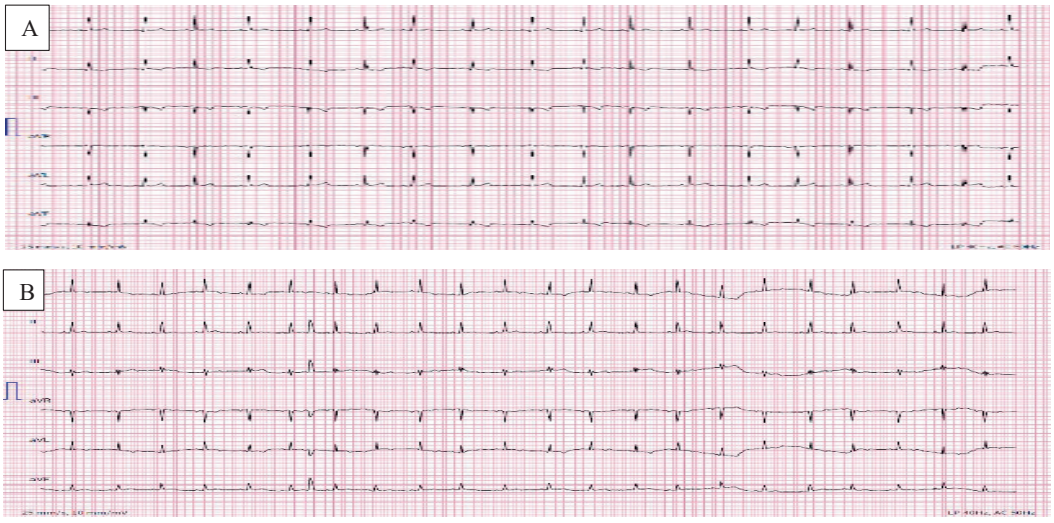


Figure 1 (A-B). The 6-lead electrocardiogram, speed 25 mm/s, amplitude 10 mm/mV. (A) performed on the first day showed 140 bpm, sinus rhythm with occasional premature ventricular contractions, and normal mean electrical axis, and (B) achieved two months after the initial presentation reviled a sinus rhythm, 110 bpm, without ventricular premature complexes

Thoracic radiographs indicated cardiac silhouette enlargement and moderate diffuse interstitial pattern in the lungs accentuated in the ventral area (Figure 3A). The Doppler ultrasound examination, which focused on the descending aorta, did not reveal any thrombus or ascites (Figure 2). A Vivid T8 ultrasonic machine (General Electric Healthcare Ultrasound) equipped with a multifrequency array transducer (2 to 7 MHz) was used for the cardiac examination. The cat was placed on the right and, afterward, on the left lateral recumbency and was examined without sedation. The same operator performed the ultrasound and the follow-up examinations using a best-practice scan of the level recommended by ACVIM to evaluate the cat's condition.

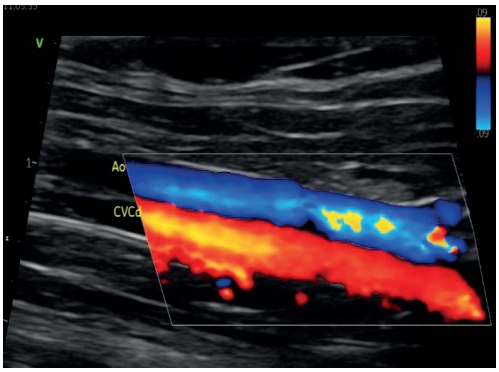


Figure 2. Abdominal ultrasound focused on the descending aorta until the trifurcation in the iliac arteries to visualize the presence of a thrombus. The Color Doppler flow was laminar and filled the lumen completely. No sign of thrombus or spontaneous contrast was visible

Echocardiography revealed moderate diffuse symmetric interventricular septal and left ventricular free wall thickening of 7.0 mm with a standard range of less than 5 mm (Figure 4A). A leading edge-to-leading edge technique was used in three cardiac cycles to determine the ventricular wall thickness during end-diastole in the long and short axes. (Boon, 2011) (Figure 4A).

The ratio between the end-systolic left atrial and end-diastolic aortic dimensions (LA/Ao) was determined in 2D in a transverse view through an inner edge-to-inner-edge technique, according to the Rishniw method. The measurement was taken from the prime diastolic frame, with the aortic valve closed, and was found to be elevated at 2.2, compared to the normal range of less than 1.6 (Rishniw et al., 2000) (Figure 5A). This ratio was obtained with a value of 2.64 in M-Mode. The left atrial fractional shortening (LAFS%) was calculated using the diastolic and systolic left atrial dimensions and was 4.5 %, below the usual 18-38% (Bussadori, 2023) (Figure 6A).

The left atrial anteroposterior diameter (LAD) was measured in a four-chambered view, inner edge to inner edge from the middle of the interatrial septum wall to the posterior free wall measured at end-systole just prior to the mitral valve leaflets opening (Smith, 2012). A value of 2.2 cm was obtained, higher than the standard measurement of less than 1.6 cm.

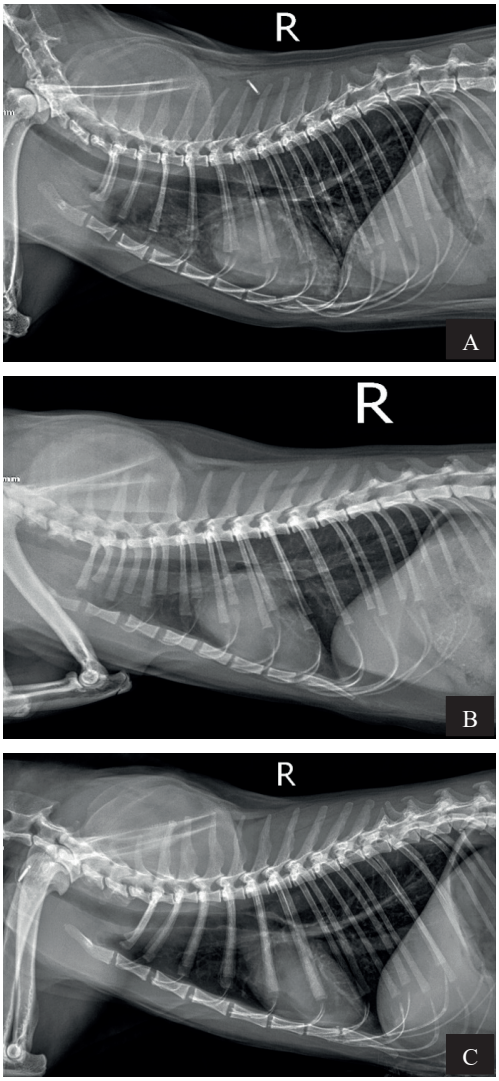
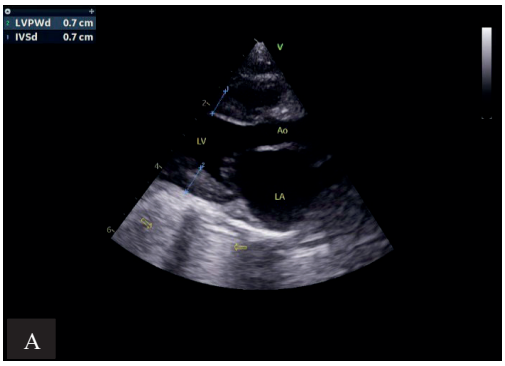
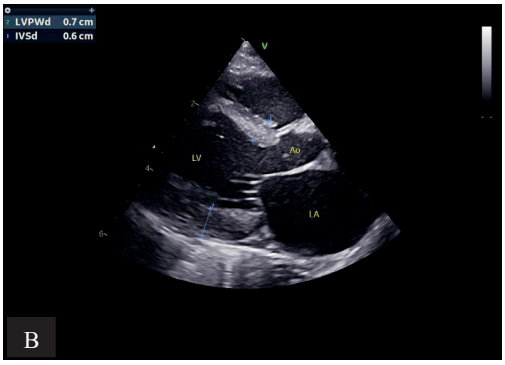


Figure 3. Thoracic radiography in right latero-lateral comparative view in day 0 (A), 48 h lateral (B), and after two months (C). (A) showed increased cardiac silhouette and moderate interstitial pulmonary pattern accentuated in the ventral field; (B) revealed the pulmonary area without infiltration, the cardiac silhouette mildly enlarged, and (C) disclosed a normal heart size and lungs without infiltrates





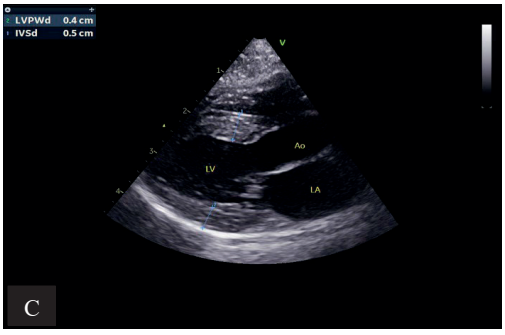
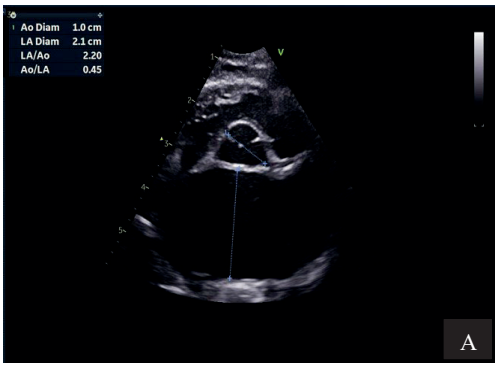
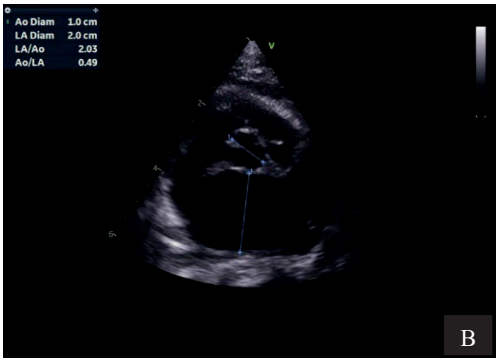


Figure 4 (A-C). Right parasternal long-axis "five-chamber view": (A) at initial presentation, (B) 48 h later, and (C) after 60 days. The initial echocardiographic image showed left atrial enlargement, increased interventricular septum wall, and left ventricular free wall thickness. Note the presence of the "B lines" (arrow) in (A), decreased after 48h (B), and normalization of myocardial thickening (left ventricular septum at end-diastole 5.0 mm, left ventricular posterior wall at end-diastole 4.0 mm, with a structural normal heart two months later (C)





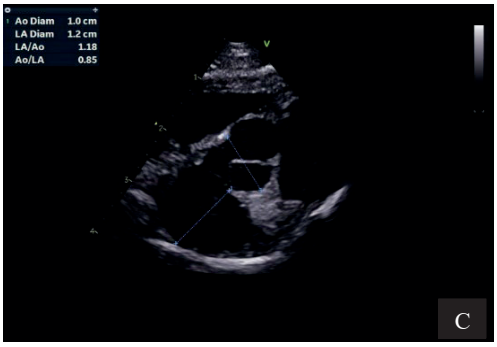


Figure 5. Right parasternal short-axis view at the aortic root level. Comparative B-Mode measurement of LA/Ao ratio in day 0 (A), day 2 (B), day 60 (C). Note the reduction of the left atrium size in (B, C)

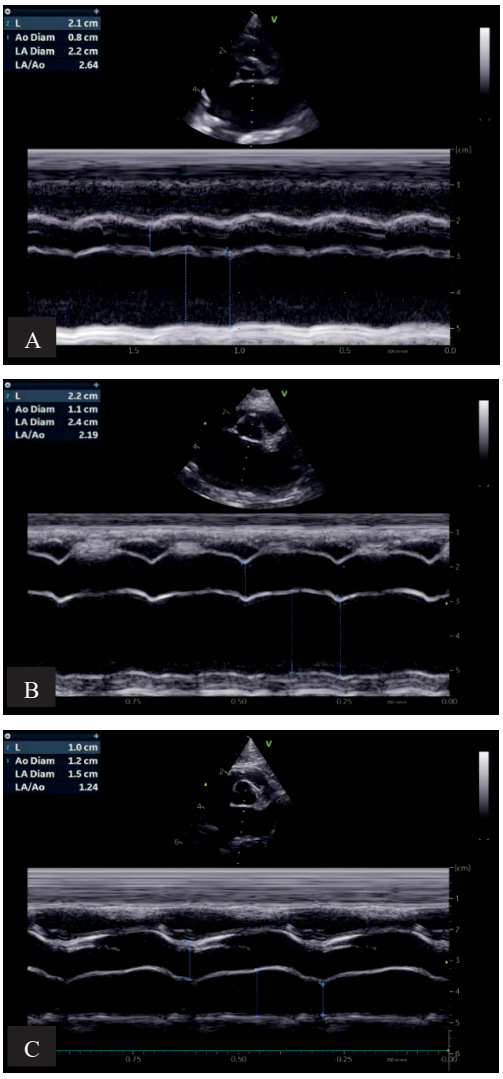


Figure 6. Right parasternal short-axis view at the aortic root level. Comparative LA/Ao ratio M-Mode measurements: day 0 (A), day 2 (B), day 60 (C). Note the reduction of the left atrium size and the improvement of the LAFS % in (B, C)

Left ventricular fractional shortening (LVFS%) was assessed using M-Mode in the "mushroom" view and found to be within the normal range (43%, standard references 30-50%) (Bonagura et al., 2000) (Figure 7A).

The systolic cranial motion of the mitral valve (SAM) is identified as the displacement of the cranial margin of the mitral valve leaflet towards the left ventricular outflow tract during systole. It causes turbulent flow, leading to obstruction and mitral regurgitation. During the examination, SAM's intermittent presence accentuated stress tachycardia was observed (Fuentes et al., 2020).

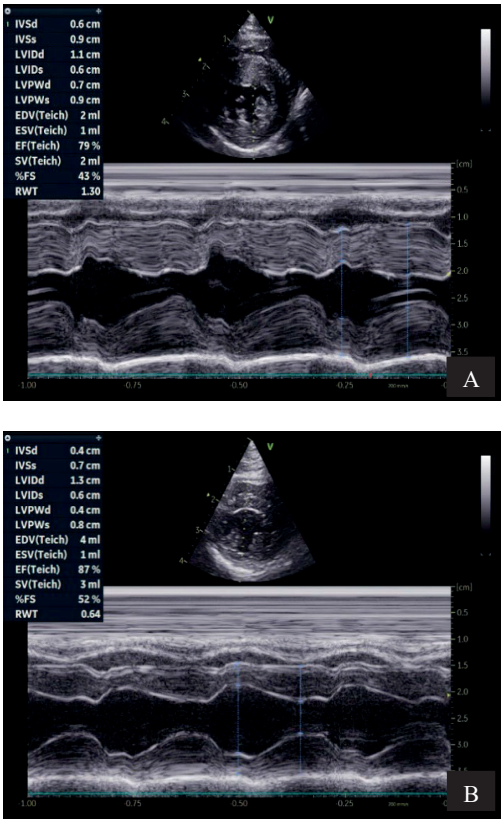


Figure 7. M-Mode measurement in right parasternal short axis view at the level of papillary muscles in diastole. (A) image obtained at the presentation and (B) obtained approximately two months after the case presentation. (B) Compared to (A), note the reduction of the interventricular septum thickness and the left ventricular free wall

The left ventricular outflow tract peak velocity was 0.8 m/s, increased on stress and visible on pulse-wave Doppler.

During the examination, spontaneous echocardiographic contrast ("smoke") or a thrombus was evaluated in the right and left parasternal four-chamber view, short-axis view at the heart base, and oblique long-axis view optimized for the left auricle. The last view is suitable for assessing the risk of thromboembolism, measuring the blood flow velocity in the left appendage. In our case, the emptying velocity was 0.16 m/s, the filling velocity was 0.18 m/s and a velocity below 20

cm/s indicated an increased risk of thromboembolic disease (Schober et al., 2006).
The diastolic function was assessed using trans-mitral flow velocities, isovolumic relaxation time, and tissue Doppler imaging.
In the left standard four chambers, the peak velocity of early diastolic trans-mitral flow showing a restrictive profile on the pulsed wave was 1.58 m/s with a deceleration time of 120 msec, and atrial contraction A-wave velocity was 0.46 m/s (Figure 8A).
The isovolumic relaxation time (IVRT) was less than 19 msec in the left apical five chambers, shorter than the standard 34-56 msec range. It was assessed with the gate volume positioned between the left ventricle inflow to record the onset of the E wave and the outflow tract to record the closure of the aortic valve (Schober et al., 2015) (Figure 8A).

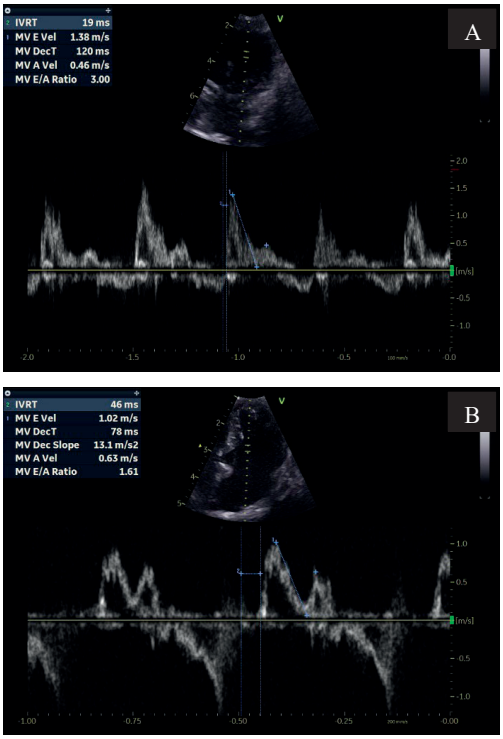


Figure 8. Echocardiographic images from initial presentation (A) revealed diastolic disfunction with a restrictive pattern. Revaluation after 6 weeks (B) revealed normal diastolic function with decreasing in E wave and increased IVRT

Tissue Doppler Imaging (TDI) was used to measure the velocity in early (E' wave) and late diastole (A' wave) by placing a pulsed wave gate at the annulus of the lateral mitral valve. In this case, the TDI showed decreased velocity in both waves, indicating a restrictive diastolic pattern (Gavaghan et al.,1999).
The echocardiographic findings were compatible with a diagnosis of hypertrophic cardiomyopathy and secondary congestive heart failure.
The cat was hospitalized and given oxygen therapy to stabilize its condition. The medications administered were Furosemide (2 mg/kg/6 h), Butorphanol (IV 0.2 mg/kg every 8 hours), Clopidogrel (18.75 mg/cat orally at 24 hours), and Pimobendane (0.125 mg/kg orally at 12 hours) due to low systolic blood pressure (90 mmHg).
This approach resulted in progressive clinical improvement within 48 hours. The patient became clinically stable; radiography showed no sign of alveolar or interstitial pattern (Figure 3B), and echocardiography measurements were ameliorated (Figures 4-8B). The cTnI level was also notably reduced (1.7 ng/ml).
The patient was dismissed home with oral medication: Clopidogrel 18.75 mg/day and Torsemide 0.1 mg/kg q24 h. The owner was recommended to monitor the cat's resting respiratory rate daily and schedule a follow-up appointment for 60 days.
The patient was examined two months later, and no abnormalities were found. Electrocardiographic, radiological, and cardiac ultrasound aspects and cTnI concentration were normalized (Figure 1B, Figures 3-8C). Clopidogrel and Torsemide was discontinued.
The owner informed us of the cat's progress via phone call six months later, and it was found that the cat showed no symptoms of illness, and its resting respiratory rate was less than 20 rpm.
After conducting a series of tests, including physical examination, laboratory investigations, chest radiographs, electrocardiogram, Doppler-focused point of care abdominal ultrasound, and echocardiogram, and following the patient's evolution, the primary diagnosis was transitory myocardial thickness.

Table 1. Evolution of the echocardiographic measurements at presentation (day 0) and follow up echocardiographic examinations (Selective two-dimensional measurement)

Day	IVSd	LVFWd	LAD	LA/Ao M-Mode	LAFS %
0	7	7	2.2	2.64	4.5%
2	6	7	18	2.22	8.3%
60	5	4	12	1.24	33%

Abbreviations: IVSd: inter-ventricular septum thickness in diastole in long axis; LVFWd: left ventricular wall thickness in diastole in long-axis. LA/Ao: right parasternal short-axis left atrium-to-aorta ratio; LAD: right parasternal four-chamber long-axis maximal inner diameter left atrium; LAFS%: left atrial fractional calculated in shortening in short axis left atrium-to-aorta.

Table 2. Evolution of the echocardiographic measurements at presentation (day 0) and follow up echocardiographic examinations (Selective Doppler measurement)

Day	E	A	E/A	IVRT
0	1.58	0.46	3.0	19
2	1.3	0.52	2.5	26
60	1.02	0.63	1.61	46

Abbreviations: E: early diastolic filling; E/A early diastolic filling to atrial contraction ratio; E': early diastole in TDI; IVRT: isovolumic relaxation time.

RESULTS AND DISCUSSIONS

Transient myocardial thickening (TMT) is a condition that initially appears similar to HCM. However, cats with TMT experience a reverse remodeling of the heart that leads to normal cardiac morphology and functionality, comparatively to familial/genetic HCM, which is typically associated with a poor long-term prognosis.

In cats, an HCM phenotype has been associated with hypertension, endocrine disorders (hyperthyroidism and acromegaly), dehydration (pseudo-hypertrophy), and infiltrative disease (lymphoma) and especially in young cats with transient myocardial thickness and myocarditis (Bond et al., 1988; Campell et al., 2007; Carter et al., 2008; Myers et al., 2014).

In humans, TMT was associated with myocarditis, Takotsubo cardiomyopathy, and amyloidosis. In the first two conditions, the increased ventricular wall thickness correlates with myocardial edema; in the last one, it is secondary to the intracellular accumulation of metabolic products. The number and dimension of myocytes and the interstitial space volume influence the myocardial wall's thickness (Fujiwara et al., 1983; Kaltenbach et al., 2008).

In hypertrophic cardiomyopathy, left ventricular wall thickness is mediated to myocyte hypertrophy and interstitial fibrous connective tissue comparatively to transient myocardial thickening caused by interstitial infiltration of proteins, cells, or fluid (myocardial edema) (Matos et al., 2018).

A retrospective study showed that cats diagnosed with transitory myocardial thickness were younger than those diagnosed with hypertrophic cardiomyopathy, with a median age of 1.7 versus eight years (Matos et al., 2017).

Certain criteria need to be met to consider a cat affected by TMT. These included undergoing at least two cardiac ultrasounds, with the initial one revealing an increase of the interventricular septum and left ventricular free wall diameter in diastole and left atrial dilatation. Follow-up echocardiographic tests were also required after a period to demonstrate normalization of these parameters.

It is recommended that the circulating levels of cTnI be tested in these cases, as they tend to be severely elevated, and then retested during the follow-up appointment to observe any reduction in cTnI levels compared to the initial evaluation. Troponins are released in the bloodstream when there is damage to cardiomyocytes, but without differentiate between primary cardiac and non-cardiac diseases involving heart damage, such as renal disease, hyperthyroidism, and anemia (Porciello et al., 2008; Sangster et al., 2014; Lalor et al., 2014).

Troponin I is veterinary medicine's most sensitive and commonly used troponin (Borgeat et al., 2015). There is a correlation between cTnI concentrations and the severity of heart disorders, and it has helped estimate the prognosis (Langhorn et al., 2014). Troponins can help differentiate between hypertrophic cardiomyopathy and myocarditis-induced thickening despite possible overlap in troponin values (Matos et al., 2018).

After a cardiac event, TnI plasma concentrations increase and peak within 24 hours. They may remain elevated for up to a week after the causative factor has been triggered.

In our case, on day 0, the level of cardiac troponin I in the blood was more than 20 ng/ml. After 48 hours, it decreased to 1.7 ng/ml and

continued to decrease to less than 0.06 ng/ml at two months control.

Another criterion that could be considered is the occurrence of antecedent stressful events, such as exposure to general anesthesia. A retrospective study suggests that 71% of cats confirmed with TMT had experienced stressful events before presenting with congestive heart failure. In comparison, only 29% of HCM cases had a history of such events (Matos et al., 2017). Another retrospective study on cats during necropsy described stressful events before the development of CHF in 75% of young cats with endomyocarditis (Stalis et al., 1995). Toby experienced dyspnea that worsened 48 hours before the emergency consultation, which occurred one week after their general anesthesia. Hypersensitivity drug reactions have been described as a cause of myocarditis in humans, which could be a potential explanation for cats since they also receive certain drugs during anesthesia induction (Kuchynka et al., 2016).

In literature, infective agents described in association with feline myocarditis and TMT were bacteria *(Bartonella henselae, Streptococcus canis),* viruses (feline coronavirus, feline immunodeficiency virus, panleukopenia virus), and parasites (*Toxoplasma gondii, Sarcocystis felis, Hepatozoon silvestris)* (Elsheikha et al., 2006; Matsuu et al., 2007; Simpson et al., 2005; Rolim et al., 2016; Kegler et al., 2018; Joseph et al., 2018; Ernandes et al., 2019; Romito et al., 2022). In the case presented, the cat was vaccinated against panleukopenia and tested negative for FIV antibodies. No further tests were done.

Although the exact cause of this condition in this species is not yet understood, it is believed that myocardial edema with inflammatory cells could be the most likely explanation. TMT may be considered a form of myocarditis, similar to what is observed in humans, and the primary cause of reverse remodeling of TMT could be transient interstitial infiltration, which is also a common characteristic of myocarditis (Hauser et al., 1983; Hiramitsu et al., 2001; Zagrosek et al., 2008; Radovanovic et al., 2022). Another possibility is that the myocardial changes could be a result of a catecholamine stream caused by emotional or physical triggers like "stress-induced cardiomyopathy", "broken-heart syndrome", or "Takotsubo cardiomyopathy".

CONCLUSIONS

Currently, limited research is available on this medical condition in cats, with only a few published studies consisting of small case series. As a result, our current knowledge of its etiology, epidemiology, clinical features, therapeutic options, and prognosis is narrow.

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ASSESSMENT OF HEAVY METALS AND TRACE ELEMENTS IN HAIR SAMPLES FROM CATS WITH GASTROINTESTINAL LYMPHOMA

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Abstract

The link between heavy metals and gastrointestinal lymphoma in cats is an area of growing concern among researchers and veterinarians. The present study assessed the concentrations of some heavy metals and trace elements in cats with gastrointestinal lymphoma, by hair sample analysis, and compared them with the elements' concentrations in clinically healthy cats. Hair samples were collected from clinically healthy cats (n = 10) and cats suffering from gastrointestinal lymphoma (n = 10). The samples underwent wet mineralization (with HNO_3 and HCl), with heavy metals and trace elements being evaluated by ICP-MS analysis. Statistical analysis was done using SPSS software. Heavy metals and trace elements were generally higher in cats with GI lymphoma. The median concentrations of Cr, As, and Se in cats with GI lymphoma were significantly higher ($p < 0.05$) compared to clinically healthy cats. Although this research found significantly higher concentrations of Cr in cats with gastrointestinal lymphoma, available studies present either no or weak evidence indicating that Cr can lead to GI lymphoma. Further research is needed to better understand the causes of this type of neoplasm in cats.

Key words*: cats; hair; heavy metals; trace elements; ICP-MS.*

INTRODUCTION

Lymphoma is reported to be the most commonly diagnosed neoplasm in felines and also represents the most common gastrointestinal cancer in both dogs and cats (Darie et al., 2023; Holland, 2020; Richter, 2003). The association between lymphoma and Feline Immunodeficiency Virus (FIV) and Feline Leukaemia Virus (FeLV) infections in cats is well established. Studies showed that cats infected with FIV have a risk of almost 6 times higher than FIV-negative cats to develop lymphoma, or that lymphoma was diagnosed in 21% of cats infected with FIV, the most common location being the gastrointestinal tract (Grover, 2005; Richter, 2003). Another study detected FeLV viral nucleic acids in over 60% of cats with gastrointestinal lymphoma using PCR (Feder & Hurvitz, 1990). However, due to development and implementation of FeLV vaccination over the years, the incidence has decreased, with alimentary lymphoma being now considered the most common type, arguing that mucosa-associated bacteria or changes in the diet or the environment may be involved in the etiopathogenesis (Hoehne et al., 2016).

Environmental pollution is undeniable, with the 20th century's increase in industrial activity, pollution occurring especially with heavy metals. The bioaccumulation of these toxic elements can cause harmful consequences in various tissues and organs, including carcinogenic effects, potentially triggering cellular changes that promote cancerous growth. Heavy metal exposure is associated, among other types of cancers, like the ones targeting the liver, kidneys, lungs, or urinary bladder, with gastrointestinal malignancies (Aalami et al., 2022; Badea et al., 2023; Morris & Dobson, 2001; Tabrez et al., 2014).

There are numerous studies that have explored the hypothesis of using animal hair as a bioindicator of environmental pollution (Badea et al., 2017; Goran et al., 2021; Kozak et al., 2002; Skibniewska et al., 2011; Skibniewski et al., 2013).

The present research assessed the levels of some heavy metals and trace elements in cats with gastrointestinal lymphoma, by hair sample analysis, and compared them with the

elements' concentrations in clinically healthy cats. Understanding and mitigating heavy metal exposure is crucial for feline health and cancer prevention.

MATERIALS AND METHODS

Hair was sampled from cats suffering from intestinal lymphoma (study group; n = 10) and clinically healthy cats (control group; n = 10). The diagnosis of intestinal lymphoma was confirmed in all patients using histopathological examination. Hair samples were collected from the flank area, packaged in envelopes and labelled. Polypropylene tubes were used to weigh 0.5 g of each hair sample with the help of an analytical scale. Organic matter disintegration was carried out by wet mineralization at room temperature, with the addition of 5 ml HNO_3 and 1 ml HCl in each tube. Mineralization was complete after two weeks, after which ultrapure water was added up to a total volume of 10 ml in each sample. The concentrations of Pb, Ni, Cr, As, Se, Cd, Hg were dosed using inductively coupled plasma mass spectrometry (ICP-MS). Statistical analysis implied the use of the Mann-Whitney test to evaluate potential differences in elements' concentrations between groups.

RESULTS AND DISCUSSIONS

The median concentrations of Pb, Ni, Cr, As, Se, Cd, Hg (ppb) of the study group and control group, together with the results of the statistical analysis, are presented in Table 1. Median concentrations (ppb) of Pb and Ni are presented in Figure 1, median concentrations of Cr, As, and Se in Figure 2, and median concentrations of Cd and Hg are shown in Figure 3.

Table 1. Pb, Ni, Cr, As, Se, Cd, Hg median concentrations (ppb) in the study and control groups

	Study group	Control group
Pb	98.12[a]	57.46[a]
Ni	91.67[a]	82.10[a]
Cr	1134.35[a]	940.65[b]
As	248.90[a]	137.63[b]
Se	474.11[a]	378.13[b]
Cd	12.78[a]	16.33[b]
Hg	5.11[a]	8.19[b]

Values with different superscripts between columns in a row vary significantly at $p < 0.05$.

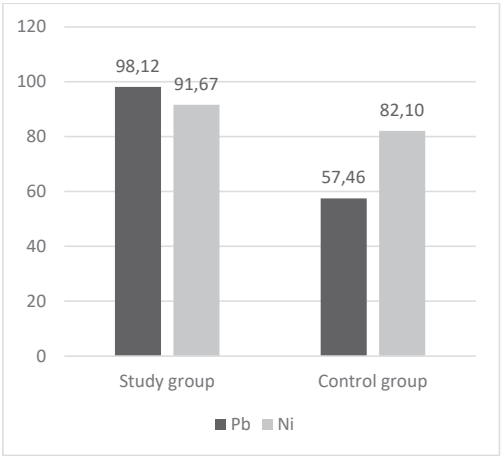

Figure 1. Median Pb and Ni concentrations in the study group and the control group (ppb)

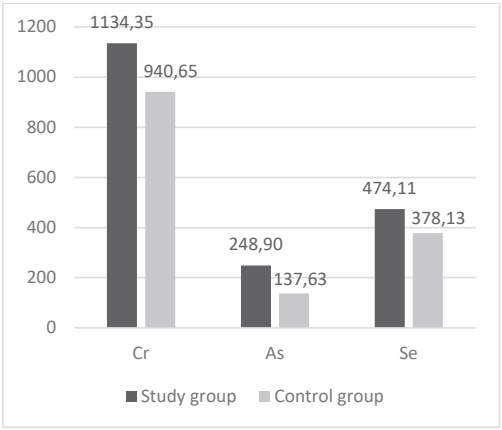

Figure 2. Median Cr, As and Se concentrations in the study group and the control group (ppb)

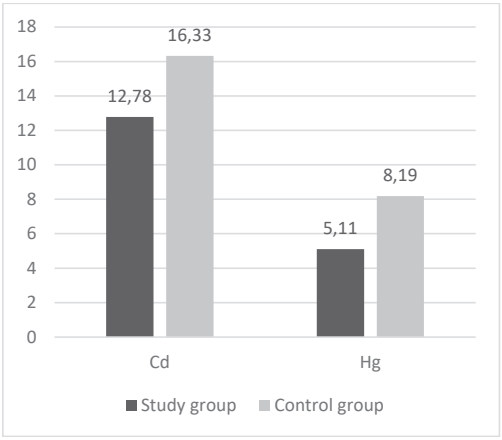

Figure 3. Median Cd and Hg concentrations in the study group and the control group (ppb)

The present study found median concentrations of Pb of 98.12 ppb in the study group, compared to 57.46 ppb in the control group, the difference having no statistical significance ($p > 0.05$). Benderli Cihan et al. (2011) evaluated some elements' concentrations in hair samples of breast cancer patients and clinically healthy humans, and found 136 ppb in breast cancer patients compared to 6196 ppb in healthy humans' hair. Higher Pb levels in the control group were also found in a study assessing heavy metal levels in dogs with mammary neoplasms, with 740 ppb in hair samples from dogs with mammary cancer and 1250 ppb in clinically healthy dogs (Badea et al., 2018).

The median concentrations of Ni were 91.67 ppb in the study group, and 82.10 ppb in the control group, but the difference was not statistically significant ($p > 0.05$).

The median concentrations of Cr were highly significantly different between the groups ($p < 0.01$), with a higher concentration in the study group (1134.35 ppb) compared to the control group (940.65 ppb). Reddy et al. (2004) analyzed trace elements in human cancerous intestinal tissue samples by PIXE technique, finding that the concentrations of Cr and Ni are higher in the neoplastic tissue of the intestine than those observed in the normal tissue.

The study group had statistically significant ($p < 0.05$) higher median concentrations of As (248.90 ppb) compared to the control group (137.63 ppb). Aalami et al. (2022) mentions arsenic exposure being associated with human lymphomas. Badea et al. (2018) found 840 ppb As in the hair of dogs with mammary adenocarcinomas; the level of As in clinically healthy dog's hair was below the method's detection limit.

The median concentrations of Se were 474.11 ppb in the study group, and 378.13 ppb in the control group, the difference being statistically significant ($p < 0.05$). Several researches showed that improper Se status is associated with long term health effects in dogs, such as mammary and prostate cancers (Fico et al., 1986; Waters et al., 2005).

The control group registered higher levels of Hg (8.19 ppb) than the study group (5.11 ppb), the difference being statistically significant ($p < 0.05$).

The control group also registered higher levels of Cd (16.33 ppb) compared to the study group (12.78 ppb), the difference being statistically significant ($p < 0.05$). Byrne et al. (2013) showed that Cd forms a high-affinity complex with estrogen receptors in the mammary gland in patients with breast cancer, suggesting an accumulation of Cd in the tissue rather than in the hair, potentially providing an explanation for the findings of the present research, since Cd's carcinogenic effects are also very well known. Cd is a potent carcinogen for humans, preferentially causing gastro-intestinal, lung, and prostate cancers (Tabrez et al., 2014).

CONCLUSIONS

Pb median concentration was higher in cats with intestinal lymphomas than in clinically healthy cats. Even if the difference is not statistically significant, it is consistent with current knowledge regarding Pb carcinogenicity.

Hg and Cd are also known carcinogens, their concentrations being however lower in the hair of cats with intestinal lymphoma compared to the controls; other studies focusing on other types of cancers showed the affinity of some heavy metals for malignant tissues, opening possibilities of further research in the present study's context as well.

As is known for inducing lymphomas in humans, which is consistent with the findings of the present research, cats with intestinal lymphomas having higher As levels compared to controls.

The scarce information regarding heavy metal and trace elements levels in cats, or animals in general, with lymphomas leaves room for continuing the research in this field, for a better understanding of the implications of potentially toxic elements in this pathology, with hope of using the findings of future studies in much needed cancer prevention.

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CLINICAL AND ULTRASONOGRAPHIC FINDINGS IN THE GALLBLADDER MUCOCELE OF THE DOG

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Abstract

Gallbladder mucocele is a common extrahepatic biliary disease in dogs, often associated with high morbidity and mortality. The aim of this retrospective study was to describe the results of ultrasonographic examination in a series of cases with mucoceles of the gallbladder in dogs. The study included 18 small breed dogs, 7 males and 11 females, aged between 7 and 18 years (mean age = 11.67 years). Eleven of the 18 dogs (61.11%) were symptomatic and 7 dogs (38.89%) were asymptomatic.Ultrasonographic examination revealed an oversized gallbladder, showing an echogenic immobile content with a different appearance. Based on the ultrasonographic images the following prevalence was found: type I - 2 cases, type II - 4 cases, type II - 3 cases, type IV - 5 cases, type V - 4 cases. Type VI has not been identified. Also, gallbladder wall rupture was not observed in any of the cases examined. In conclusion, ultrasonography is the standard imaging method for the diagnosis of gallbladder mucoceles in dogs, revealing the presence of an enlarged gallbladder with an immobile bile pattern and variable appearance.

Key words*: dogs, gallbladder mucocele, ultrasonography.*

INTRODUCTION

The liver is the main gland of the digestive tract, which also has the role of synthesizing bile that is stored in the gallbladder (Gookin et al., 2023). As a result of mechanisms not yet fully understood, the gallbladder epithelium secretes an excessive amount of abnormal mucus, which accumulates in the gallbladder and undergoes continuous changes, resulting in a condition called gallbladder mucoceles (Kesimer et al., 2015; Mizutani et al., 2017; Codreanu & Popa, 2018). The accumulation of inspissated mucus in the lumen of the gallbladder can lead to its distention, accompanied by obstruction of the extrahepatic bile ducts and the onset of cholecystitis (Besso et al., 2000; Codreanu, 2010; Kesimer et al., 2015; Smalle et al., 2015; Rogers et al., 2020; Itoh et al., 2022; Jaffey et al., 2022). In addition, as a result of gallbladder distention, necrosis and/or rupture of the gallbladder wall may occur, leading to systemic inflammatory response syndrome and death of the patient (Smalle et al., 2015; Fuerst et al., 2019; Itoh et al., 2022). The death rate varies between 7% and 45% (Jaffey et al., 2019).

The aetiology and pathogenesis of the disease are not known and it is considered a multifactorial condition, including genetic and metabolic influences (Choi et al., 2014; Smalle et al., 2015; Fuerst & Hostnik, 2019; Jaffey et al., 2022). Among the risk factors for gallbladder mucoceles, hyperadrenocorticism, hypothyroidism, dyslipidemia, increased serum leptin concentration have been suggested (summarized in Butler et al., 2022). An increased frequency of this condition has been found in middle-aged and older dogs of pure breeds such as Shetland sheepdogs, Cocker Spaniels, Toy Poodles, Border terriers, Pomeranians, Chihuahuas, Miniature Schnauzers, Shih-tzu, Maltese and Beagles, as well as their mixes (Besso et al., 2000; Choi et al., 2014; Itoh et al., 2022; Jaffey et al., 2022). Although the disease may progress asymptomatically (Besso et al., 2000; Pike et al., 2004; Choi et al., 2014), the majority of patients present with a varied and often non-specific clinical picture, including anorexia, vomiting (following extrahepatic bile duct obstruction, or perforation/rupture of the gallbladder wall and peritonitis), hyperthermia and abdominal pain

(Worley et al., 2004; Choi et al., 2014; Smalle et al., 2015). Ultrasonographic examination highlights specific aspects of immobile bile material in the lumen of the gallbladder, which may show a stellate pattern or a fine striated pattern (so-called "kiwi fruit like" pattern)(Choi et al., 2014; Jaffey et al., 2022; Codreanu et al., 2022; Tudor & Codreanu, 2023), the definite diagnosis being established by histopathological examination (Besso et al., 2000). The aim of this retrospective study was to determine the types of gallbladder mucoceles in ultrasonographically examined dogs in order to guide therapeutic management.

MATERIALS AND METHODS

The medical records of dogs examined ultrasonographically in Link-Vet Veterinary Clinic, Bucharest, from 2017 to 2022 were retrospectively analyzed. Data retrieved from medical records referred to breed, age, sex, clinical signs and ultrasound examination result. At the time of presentation to the clinic, consent was obtained from the owners for the application of clinical and imaging assessment techniques to canine patients, as well as permission to use data from the dogs medical records. Ultrasonographic examination was performed using a General Electric Logiq S8 XD Clear 2.0 ultrasound machine, monitoring gallbladder size, gallbladder wall echogenicity and thickness, echogenicity and mobility of gallbladder contents, and distribution pattern of gallbladder contents. The presence of gallbladder mucoceles was established when the gallbladder was found to contain immobile material that did not move with gravity and had a fine striated and/or stellate appearance (Besso et al., 2000; Choi et al., 2014; Jeffey et al., 2022; Codreanu et al., 2022).

RESULTS AND DISCUSSIONS

Between 2017 and 2022, 995 dogs were registered, of which 294 had gallbladder disease. Of the dogs with gallbladder disease, 18 dogs were diagnosed with mucoceles (6.12%; 18/294). They were represented by 7 males (2 male-entire and 5 male-spayed) and 11 females (9 female-entire and 2 female-spayed), ranging in age from 7 to 18 years (mean age = 11.67 years). Breeds represented were mixed breeds (n=12), Beagle (n=2), Yorkshire (n=2), Miniature Schnauzer (n=1) and Pekingese (n=1) Seven of the 18 dogs were asymptomatic (38.89%), while 11 dogs (61.11%) were symptomatic, exhibiting vomiting (n=11), anorexia (n=8), hyperthermia (n=6), abdominal pain (n=7) and jaundice (n=4). Patients were diagnosed with gallbladder mucoceles by utrasonography, which is the current standard for diagnosis of this condition (Fuerst & Hostnik, 2019). As a result, ultrasonographically, the gallbladder was found to be distended, which showed an immobile bile content with a different appearance, on the basis of which each case was classified into one of the six known types, using the previously established classification (Besso et al., 2000). In 2 dogs (11.11%), a hyperechogenic, immobile, fine-structured content was found in the gallbladder lumen and classified as type I (Figure 1).

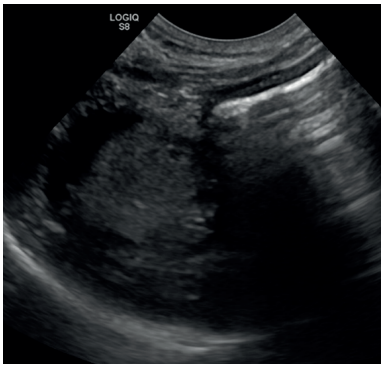

Figure 1. Type I, the gallbladder has an echogenic, immobile, non-gravity-independent bile content with a hypoechoic border

Four dogs (22.22%) were classified as type II (Figure 2), in which the biliary material has a hyperechogenic appearance with extensions adhering to the gallbladder wall giving a partially stellate appearance and a hypoechoic appearance at the periphery. In 3 dogs (16.67%) type III was identified (Figure 3), characterized by the accumulation of a viscous, hyperechogenic, stellate bile material on the hypoechoic background of the gallbladder, the so-called stellate pattern.

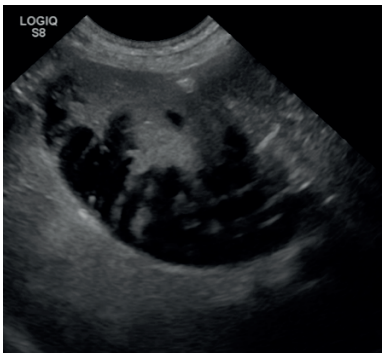

Figure 2. Echogenic biliary material shows a partially stellate pattern with adhesions to the gallbladder wall - type II

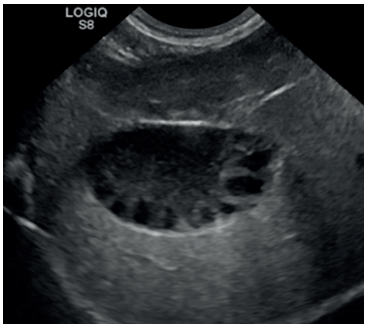

Figure 3. In the lumen of the gallbladder the echogenic, immobile bile content has a stellate appearance - type III

In 5 dogs (27.78%), type IV (Figure 4) was found, characterized by a combination of a stellate pattern (in which echogenic bile material located in the lumen of the gallbladder shows peripheral elongations, accompanied by peripheral hypoechogenicity) and a fine striated pattern (with thin hypoechogenic striations on the residual echogenic bile material), giving a kiwi fruit-like appearance in section. In 4 dogs (22.22%), type V was identified (Figure 5), in which the viscous and immobile contents of the gallbladder present the image of a kiwi fruit per section, with echogenic remnants. Type VI was not identified. Also, no rupture of the gallbladder wall was observed in any of the cases examined.

Mucocele is an abnormal accumulation of inspissated mucus produced in excessive amounts by the epithelium of the gallbladder wall, with increased viscosity, resulting in enlargement of the gallbladder (Choi et al., 2014; Codreanu & Popa, 2018; Tudor & Codreanu, 2023).

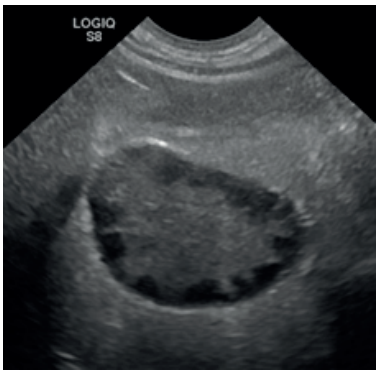

Figure 4. Type IV - gallbladder material shows a combination of stellate pattern and kiwi fruit-like pattern on section, gallbladder wall thickened

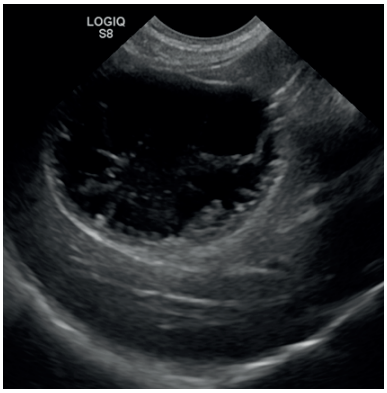

Figure 5. The bile material shows a characteristic kiwi fruit-like appearance in section, with echogenic debris

The aetiopathogenesis of gallbladder mucoceles is still uncertain and several hypotheses have been suggested (Choi et al., 2014; Jaffey et al., 2019). Among others, it has been suggested to be the consequence of a chronic inflammatory process of the gallbladder, mucinous hyperplasia of the gallbladder wall epithelium, biliary stasis or obstruction of biliary structures (Besso et al., 2000; Galley et al., 2022), however, no sufficient arguments were identifies to support one or the other of the suggested hypotheses, considering it a multifactorial condition (Smalle et al., 2015; Galley et al., 2022). In addition, it has been found that bile duct obstruction (either structural or functional in nature) can cause biliary stasis to occur, but mucocele does not form when bile content stasis is caused by structural obstruction (Besso et al., 2000).

Analysis of the results obtained in this study shows the presence of gallbladder mucoceles in 1.83% (18/995) of the dogs examined during the period evaluated, a condition characterized by the presence of immobile biliary material in the lumen of the gallbladder, which distinguishes it from biliary sludge (Mizutani et al., 2017). In addition, previous studies suggest that biliary sludge may represent a stage preceding the appearance of gallbladder mucoceles (Mizutani et al., 2017; Butler et al., 2022). Gallbladder mucocele has been identified in 5 small breeds, predominantly in mixed breeds, but purebred dogs such as Beagle, Yorkshire, Miniature Schnauzer and Pekingese have also been affected, as previously reported (Jaffey et al., 2019; Jaffey et al., 2022; Itoh et al., 2022). Regarding the age of the patients, in the present study, gallbladder mucocele was diagnosed in adult dogs, the average age recorded was 11.67 years, similar to those previously published in the literature (Choi et al., 2014; Jaffey et al., 2019; Galley et al., 2022; Jaffey et al., 2022; Itoh et al., 2022).

As in the previous studies (Choi et al., 2014; Jaffey et al., 2022), The present study found that the patients evaluated were both asymptomatic (38.89%) and symptomatic (61.11%), and the clinical signs were variable, mainly represented by vomiting, anorexia and abdominal pain. Ultrasonographic examination revealed the existence of bile material in the lumen of the gallbladder, which showed different patterns, especially type IV, without detecting type VI, characterized by the fine striated appearance of all bile material. In a previous study (Choi et al., 2014), it was found that in the 43 dogs evaluated ultrasonographically all biliary patterns were identified (30.23% showed type II, 25.58% type IV, 23.26% type I, 11.63% type III and 9.30% type V) except type VI. In contrast, in another study of 216 dogs, ultrasonographic examination was performed on 185 patients and all VI types of gallbladder mucoceles were identified (29.73% type II, 23.78% type IV, 17.3% type I, 14.59%, 9.73% type III and 4.86% type VI). A possible explanation for the differences observed in the present study compared to those previously published is the different number of patients examined. Although gallbladder rupture is a common complication in dogs with gallbladder mucoceles (Choi et al., 2014; Jaffey et al., 2018; Jaffey et al., 2022), in our study this change was not recorded.

Regarding the association between the clinical status of patients with mucoceles and the ultrasonographic type of gallbladder, previous studies have suggested that patients with severe clinical signs have an increased risk of a more advanced pattern of gallbladder mucoceles (Jeffey et al., 2022), this was also observed in the present study, in which symptomatic patients showed a more advanced pattern (IV and V). Identifying changes in the gallbladder in correlation with the symptoms expressed by the patient is of significant importance, helping to determine the appropriate therapeutic conduct for each patient. Previous studies have highlighted the association of gallbladder mucoceles in dogs with increased mortality if not diagnosed early and managed appropriately (Galley et al., 2022). The present study had some limitations due to its retrospective nature and the lack of information on patients subsequent outcome.

CONCLUSIONS

Ultrasonographic evaluation revealed characteristic changes in the gallbladder, recording its distention by an immobile bile content, with a variable pattern, stellate or finely striated, resembling a kiwi fruit in section. Based on the clinical manifestations in association with the type of gallbladder pattern identified ultrasonographical, the therapeutic management was established.

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CLINICAL APPROACH AND EFFECTIVENESS OF THERAPY WITH SOME ANTIULCER DRUGS IN DIGESTIVE DISEASES IN DOGS

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Abstract

An important share in canine digestive pathology is represented by gastrointestinal ulcers of various causes. Between them, gastritis, ulcers, hypergastrinemic syndromes and last but not least the prevention and combating of the unwanted effects of non-steroidal anti-inflammatory therapy, can occur in carnivores when the protective factors are disturbed or compromised. In such situations, administration of proton pump inhibitors, H2 receptor antagonists or local antacids is routine and of undeniable utility. Our study was carried out on 12 clinical cases that presented to the veterinary clinic, dogs of different breeds and ages, both female and male, in which the therapeutic efficiency was monitored after the administration of drugs to combat acidity.
After performing the treatment, healing occurred in 91.66% of cases, failure registering 8.33% of the total.
A correct and complete clinical approach, with carefully chosen and regularly administered drugs, increases therapeutic efficacy in multicausal canine ulcer pathology.

Key words: *antiulcer drugs, digestive diseases, dogs, therapy.*

INTRODUCTION

When the protective factors of the gastric mucosa are compromised or disturbed, are created the conditions for the appearance of ulcers in animals, particularly small animals, the most affected appear to be dogs (Allen et al. 1993).
Gastrointestinal ulcers are a major health problem in dogs and a predisposing factor is the use of ulcerogenic drugs (NSAIDs, corticosteroids or gastrointestinal irritants), on one hand and stress, inflammatory diseases, even breed predispositions, on the other hand (Dobre, 2019; Dobre et al., 2019; Ghiță, 2021; Goran, 2016).
If it is damaged, the protective barrier of the gastroduodenal mucosa allows hydrochloric acid, bile acids and proteolytic enzymes to begin degrading the intestinal epithelium, disrupting the lipid membranes and initiating the inflammatory process (Blois, 2020).
Clinical signs in dogs, as in pigs, horses and zoo animals, are absent or most often non-specific, represented by vomiting, anorexia, abdominal pain and weight loss. The occurrence of this disease in pigs causes economic losses through organoleptic changes in the meat (Petcu, 2013; Petcu, 2015).
The antacids, those based on Al, Mg or Ca, are used for their effect of neutralizing acidity, reducing pepsin activity and stimulating local prostaglandin synthesis.
H2 receptor antagonists such as cimetidine and or ranitidine are used in doses of 10 mg/kg (cimetidine) and 2 mg/kg (ranitidine), twice a day, as blockers of gastric acid secretion. (Papich, 2018).
In addition, proton pump inhibitors, such as omeprazole or pantoprazole are used to interfere with the final step of acid production, with a maximum inhibitory effect in 2-4 days (Kromer et al., 2000).
The aim of the present study is to evaluate the therapeutic effectiveness of antacid drugs in ulcer disease in dogs (the dogs were diagnosed with ulcer disease on presentation to the veterinary clinic).

MATERIALS AND METHODS

The study was carried out on a number of 12 canine patients, both sexes. The patients were presented to the veterinary clinic with non-

specific digestive symptoms (vomiting, anorexia, abdominal pain, weight loss) and they underwent gastrointestinal endoscopy for the identification of ulcerative lesions (Table 1).

Table 1 Patients included in the study

ID patient	Breed	Age	Weight	Sex
1	Rotweiller	6 yo	35 kg	♀
2	Setter	4 yo	32 kg	♂
3	Jack Russel	7 yo	8 kg	♀
4	Caniche	4 yo	4.5 kg	♂
5	Ciobănesc	2 yo	41 kg	♂
6	Mixed	8 yo	14 kg	♂
7	Akita Inu	5 yo	39 kg	♂
8	Pug	6 yo	11.5 kg	♀
9	Mixed	3 yo	11 kg	♂
10	Golden Retriever	2 yo	31 kg	♂
11	Mixed	5 yo	13 kg	♀
12	Mixed	10 yo	16 kg	♀

Following the endoscopy, gastric ulcer lesions were identified (Figure 2). A perforated ulcer was identified in patient ID 6.
The patients included in the study were treated with local antacids Gaviscon oral suspension 10 ml three times a day every, and with H2 receptor inhibitors, ranitidine (Zantac) 2 mg/kg/day, respectively proton pump inhibitors (PPI), pantoprazole (Controloc) 1 mg/kg/day, according to the therapeutic scheme shown in Table 2. Were used Zantac injectable 50 mg s.a., (2 ml vials) and Controloc 40 mg s.a./vial.

Table 2. Amounts (ml/animal) of solution administered to patients taking into account body weight

Drug / ID Patient	Controloc IV	Gaviscon PO	Zantac IM
1	8.8 daily	10 tid	2.8 daily
2	8 daily	10 tid	1.3 daily
3	2 daily	10 tid	0.6 daily
4	1.1 daily	10 tid	0.4 daily
5	10 daily	10 tid	3.3 daily
6	3.5 daily	10 tid	1.1 daily
7	9.8 daily	10 tid	3.1 daily
8	2.9 daily	10 tid	0.9 daily
9	2.8 daily	10 tid	0.9 daily
10	7.8 daily	10 tid	2.5 daily
11	3.3 daily	10 tid	1.0 daily
12	4 daily	10 tid	1.3 daily

Zantac was administered IM daily, and Controloc was administered IV, in the morning after reconstitution with 10 ml of saline for injection, in amounts calculated according to body weight, as can be seen in Table 2.

Figure 1. Gastric ulcer in the cardia area in a dog

The treatment was carried out for 10 days, following the evolution of the clinical picture.
The response to therapy was monitored by improvement of clinical signs after 5 days of treatment. The treatment was continued for an additional 5 days in patients who did not respond to treatment within the first 5 days.
The effectiveness of the treatment was evaluated as reported by the therapy outcome, by sex and by age group.

RESULTS AND DISCUSSIONS

Therapy was instituted after clinical examination and diagnosis of ulcerations by gastrointestinal endoscopy, hematological and biochemical examination. Abdominal radiography was helpful only in case of perforated ulcer, which was encountered in our study only in patient ID 6.
The clinical manifestations highlighted during the clinical examination were vomiting, diarrhea, inappetence and abdominal reactivity as can be seen in Figure 2.

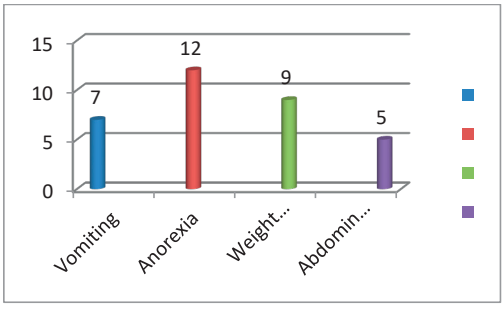

Figure 2. The main clinical manifestations in the studied animals

Regarding the gender distribution, 7 cases out of the 12 studied were represented by males,

i.e. 58%, while females were 5 of the cases presented, respectively 42% (Figure 3).

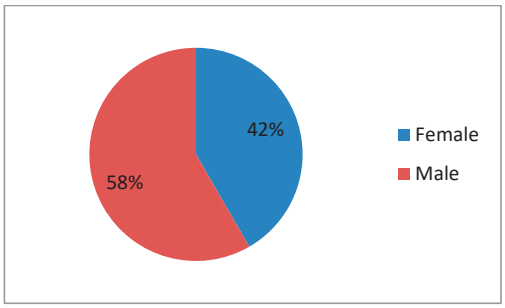

Figure 3. Incidence by gender of ulcer disease in the followed patients

Regarding the age of the patients, three of the cases fell within the age range of 1 to 3 years, 25%, six were 4 to 6 years, i.e. 50%, and three patients were over 6 years, respectively 25%, as can be seen from Figure 4.

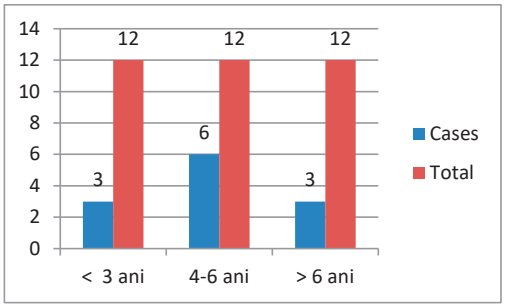

Figure 4. Distribution of cases by age category

As shown, the medication was administered daily to all patients, following their clinical evolution. For 7 of the canine patients, signs of improvement appeared beginning on the second, third day of the treatment.

Therefore, the favorable evolution led to the interruption of therapy after the 5th day of antacid medication administration. For the other 5 members of the studied group, in whom no improvement of the general condition was found, the therapy was continued for another 5 days. Patients with ID 1, 6, 7, 11 and 12 were further monitored on therapy as shown in Table 3.

Regarding the susceptibility to this disease, various factors can be invoked such as previous use of NSAIDs, genetic predisposition (in sled dogs) or poor maintenance that affects the body's resistance.

Table 3. Patients to whom therapy was continued for 10 days

ID patient	Breed	Age	Weight	Sex
1	Rotweiller	6 yo	35 kg	♀
6	Mixed	8 yo	14 kg	♂
7	Akita Inu	5 yo	39 kg	♂
11	Mixed	5 yo	13 kg	♀
12	Mixed	10 yo	16 kg	♀

We can observe that 3 of the five cases were female, that 1 patient is 10 years old or that 3 of them are of mixed race. The observations cannot represent a trend due to the small number of patients participants.

The prognosis formulated at the beginning of the treatment was reserved in the 11 cases of non-perforated ulcer diagnosed endoscopically, and severe in the patient with perforated ulcer.

In addition to specific therapy to reduce acidity, patients received supportive adjuvant therapy (fluidotherapy) and antibiotic protection in case of major gastric mucosal involvement.

Finally, the treatment yielded results in 4 more patients. Unfortunately, ID 6 patient, who initially had a perforated ulcer, died on the 8th day of treatment after surgery.

This means that in the framework of our approach, the effectiveness of the therapy is finally verified in 11 of the cases, i.e. in 91.6%, failure being represented by 1 case, respectively 8.4% (Figure 5).

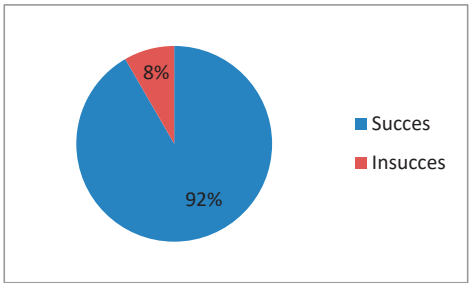

Figure 5. Rate of therapeutic success following drugs administration

In view of the almost unanimous opinion that frequent administration of antacids is disadvantageous to animals, the frequency of administration of Gaviscon every 8 hours could

be supported and appeared to count in ameliorating signs of disease.
Parenteral administration of the other drugs avoided problems such as oral administration of different drugs at different times to avoid interaction between them.
The administration of H2 antihistamines helped to protect the injured gastric mucosa, and undoubtedly contributed to supporting the prognosis, due to the increased efficacy of ranitidine compared to cimetidine and possibly also due to the prokinetic effect (Papich, 2018).
Even if more and more authors recommend the administration of PPIs twice a day, the solution selected by us (a single administration of pantoprazole daily) was not without results (Tolbert et al., 2015).
The efficiency of our therapeutic protocol has finally proven by the almost maximum proportion of cures.
Compared to the recommendation made for the duration of therapy in human medicine of 4 weeks, we appreciate as practice this approach of carrying out the therapy for a period of at least 10 days (Blois, 2020).

CONCLUSIONS

The causes of gastric ulcers appearance in dogs are various, including therapy with NSAIDs, corticosteroids, gastric irritants, stress or breed predisposition.
The therapeutic protocol included an antacid (Gaviscon), an H2 antihistamine (Ranitidine) and pantoprazole (PPI), which were administered for 10 days.
In the study, there was no higher incidence in a certain breed, sex or age group.
The therapeutic efficiency was 91.66% healing.

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HEPATIC AND RENAL IMPAIRMENT IN ANTICOAGULANT RODENTICIDE POISONING

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Abstract

This paper aims to highlight the impairment of the renal and hepatic function in anticoagulant rodenticide poisonings. The study involves 40 cases taken from the archive of the Veterinary Emergency Hospital within the Faculty of Veterinary Medicine in Cluj-Napoca between 2017 - 2022, 7 cats and 33 dogs.
This article collects important laboratory values, we elaborated a database to be able to diagnose, treat and evaluate the renal and hepatic status of these animals. For the hematology part a VetScan HM5 device was used, thrombocytopenia was found, the erythrocytes, haemoglobin and haematocrit had lower values. For the biochemistry part we used a PrimeVet device, we detected important changes in the level of glucose, lactate, total bilirubin, smaller changes in creatinine and urea. For the coagulation tests a Quickvet specialty analyser was used, the prothrombin time in 50% of the cases presented low values, 14% presented normal values, and in 36% had high values. In each case the partial thromboplastin activation time presented high values. We detected kidney insufficiency in 2 cats and 3 dogs, and liver problems in 2 cats and 8 dogs.

Key words*: rodenticide, coagulation, haematology.*

INTRODUCTION

The intoxications with first generation or second generation anticoagulant rodenticides in pets are frequently seen (Valchev et al., 2008).
The main purpose of this research is to highlight the liver disfunctions and kidney damage in case of poisonings with anticoagulant rodenticides and to establish the percentage of patients who developed liver or kidney disfunction or suffer from both at the same time.
Rodenticide anticoagulants from the first and second generation are the most used to combat rodents, these substances will affect the blood clotting processes, leading to death by internal or external haemorrhages (Gunja et al., 2011; Subban et al., 2012). The intoxicated animals will constantly lose blood in small amounts, remain conscious until death accrues, accompanied by severe pain. Although it is not known exactly how long it may take for an animal to die, as this period is highly influenced by the dose ingested by the animal and its body weight, some studies have concluded that this time is about 3 days for rats, others describe a week, and in case of mice it may last a little bit longer (Berny et al., 1995; Munday and Thompson, 2003; Wu et al. 2012; Casner, 1998; Baker et al., 2002).
These anticoagulants are also dangerous to non-targeted animals, such as wild animals and companion animals, these can consume the baits for rodents, or the poisoned animal itself. The first generation anticoagulants persists for about 7 days in an animal's organism, but

second generation anticoagulants are much stronger, persisting for about 4 weeks in the body. The mechanism of action of all anticoagulant rodenticides is through inhibition of vitamin K_1 epoxide reductase (Breckenridge et al., 1985), this will lead to coagulopathy, due to the reduction of the active forms of coagulation factors: II, VII, IX and X (Craciun, 1998). Because these factors are active in fibrin formation, they need to be able to bond calcium, which is possible if each coagulation factor present in their structure carboxylic acid. Vitamin K_1 intervenes on the coagulation factors by adding the second carboxylic acid, produce a post-translational modification of the coagulation factor protein and becomes a vitamin K_1-epoxide oxidase, during this process. Vitamin K_1 epoxide can normally be returned to its original vitamin K_1, with the help of enzymes, being a recycling of vitamin K_1.

At this stage rodenticide anticoagulants will inhibit the activity of these enzymes, resulting a decrease in the amount of vitamin K_1 and an increase in the concentration of vitamin K_1-epoxide in the hepatocytes and in the plasma. After the almost complete depletion of these factors and vitamin K_1, clinical coagulopathy occurs (Walker et al., 2012). This time interval explains the delay in the appearance of clinical signs, which can reach up to 5 days after the ingestion of anticoagulant rodenticides and can be correlated with haematological, biochemical laboratory analyses and coagulation times.

MATERIALS AND METHODS

The study involved 40 cases taken from the archive of the Emergency Veterinary Hospital of the Faculty of Veterinary Medicine in Cluj-Napoca, over a period of 5 years, between 2017 and 2022, and included 7 cats and 33 dogs.

The cats ranged in age from 3 months to 9 years, 5 of them were male and 2 females. The dogs were aged between 3 months and 11.5 years, 16 cases are male and the remaining 17 are female. Among the dogs, we had: 10 Mestizos, 4 Labradors, 3 Bichons, 2 Beagles, 2 Huskies, 3 German shepherds, a Dachshund, a Mioritic shepherd, a French bulldog, a Pincher, a Tosa inu, an Akita inu, a Hungerian vizsla, a Dalmatian, and a Golden retriever.

RESULTS AND DISCUSSIONS

In order to obtain a complete panel about this poisoning and to facilitate the work of clinicians, to make quick and correct diagnoses and efficient treatments, the data's obtained were processed statistically, and will be presented in tables, in 3 categories. We will present the general clinical signs, haematology values, biochemical data's and coagulogramma results.

Cats show mild to moderate bradycardia, with mild to severe tachypnea and the body temperature was in 33.33% low, 33.33% normal and high in 33.33% cases. The data is being arranged in Table 1.

Table 1. Clinical data in cats

Nr caz	Nr crt	Age	Sex	CRT (Sec)	Mucous membr.	FC.	FR.	Temp (°C)
4	1	9 years	M	1	Pale pink	116 ↓	40 ↑	36,4 ↓
7	2	4 years	M	-	Pale pink	-	-	39,13
13	3	3 month	M	2	Pale pink	100 ↓	30 ↑	37,0 ↓
23	4	1 year	M	-	-	-	-	-
31	5	6 month	M	3	Pale pink	136 ↓	112↑	39,9 ↑
39	6	6 month	F	3	Pale pink	-	-	40,9 ↑
40	7	6 month	F	2	Pale pink	-	160↑	38,2

In the haematology part (Table 2), the changes were not so obvious, regarding the haematocrit and the red blood cell volume, these values tend to decrease.

Table 2. The haematology values in cats

Nr. case	Nr. Crt.	WBC 10^9/L	RBC 10^{12}	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	PLT 10^9/L	MPV fL
Ref Int.		5,5-19,5	5-10	8-15	24-45	39-55	12,5-17,5	30-36	300-800	12-17
4	1	7,93	9,28	11,8	28,2	30 ↓	12,7	41,6	164 ↓	7,1 ↓
7	2	5,5	6.29	8,2	28,43	45	13	28,8 ↓	35 ↓	9,6 ↓
13	3	-	-	11,4	38	-	-	-	-	-
23	4	-	-	12,45	40	-	-	-	-	-
31	5	-	-	15,1 ↑	46 ↑	-	-	-	-	-
39	6	-	-	8,6	27	-	-	-	-	
40	7	7,37	6,54	10,1	26,58	40,6	15,5	38,2 ↑	234 ↓	9,9 ↓

The increase of the MCHC can suggest a macrocytic anaemia, and in three pacients accured thrombocytopenia as similar changes were seen in other studies too (Kohn et al. 2003). Thrombocytopenia may also be caused

by vitamin K deficiency and decreased hepatic thrombopoietin synthesis (Andrew B., 2019).
Renal function impairment is seen in 2 cats out of 7 cases, these animals are presenting a high level of urea, this can also reflect a state of malnutrition, protein-deficient diet or liver disease. Glucose is irrelevant in felines, because they stress quickly which consequently will increase the glucose level too. Sodium and potassium show a slight increase in case 23, where the urea was increased too, these values reflecting a renal insufficiency. Total bilirubin can be elevated due to liver damage (Table 3).
Most changes occur in the coagulogramm, where the prothrombin time and partial thromboplastin time are increased, due to the anticoagulant rodenticides interference with the vitamin K cycle.

Table 3. Biochemical datas in cats

Nr. caz	BUN mg/dL	Creat. mg/dL	Glu mg/dL	Na mmol/L	K mmol/L	Total bilirubina mg/dL	Acidul Lactic mmol/L
Ref	**19-34**	**0,9-2,2**	**60-120**	**146-156**	**3,7-6,1**	**0,1-1,2**	**<2,5**
4	19	1,4	136 ↑	155	3,9	0,7	1,1
7	-	1,2	101	-	-	-	-
13	9 ↓	0,6 ↓	102	146,5	4,88	2 ↑	1,8
23	126,5 ↑	-	111,75	158,5 ↑	6,27 ↑	-	1,65
31	38 ↑	1	138 ↑	154,4	4,17	0,7	11,1 ↑
39	15 ↓	0,7 ↓	146 ↑	150,8	4,29	2 ↑	1,3

Dogs presented pale mucous membranes to slight congested ones, possibly caused by haemorrhage, hypovolemia, shock or dehydration, some dogs had positive results at TFAST and AFAST, observing free fluid in the thorax and abdomen, due to internal bleeding, hematemesis or presented external haemorrhages as petechiae on the skin, vulva, and buccal cavity (Table 4).
All patients who exceeded the normal limits of respiratory rate presented tachycardia, that may be due to cardiac causes, liver diseases, parasites or could appear because of a compensatory factor in hypovolemic states, shock, or excessive effort. As respiratory signs the animals presented dyspnoea, wet rales, emetic cough or coughs.
In dogs the haematology exam (Table 5) presented low values for erythrocytes that can be caused by haemorrhages, by damage to the kidney, especially affecting the secretion of erythropoietin. Hemoglobin and hematocrit decrease in anemic states, hemorrhages, liver and kidney diseases, etc. Elevated MCHC values indicate macrocystic anemia, and thrombocytopenia due to rodenticide poisoning. The urea level was elevated in 15.78% of the dogs, and in 10%, of the examinated animals we observed increased creatinine values to.
The glucose level was highly increased, but that could be related to the treatment.
The electrolytes were decreased, Na was presented lower levels in 35% of the dogs, K in 40% was decreased.
The total bilirubin in 47% of the cases was high, and the lactic acid was increased in 52.64% of the dogs (Table 6).
In anticoagulant rodenticide poisonings the coagulogramma results are the most trustful. In our research the results showed that the prothrombin time was in 50% from the examined animals decreased, normal in 14% and increased in 36% of the dogs (Figure 1). The partial thromboplastin activation time was in 100% elevated, the lowest value started from 20 sec and the highest measured maximum values was 200 sec. In a spontaneous intoxication with bromadiolone Binev et al., 2005 reported the prothrombin time was 122 s (reference range: 12-14 s) and the activated thromboplastin time was 88 s (reference range: 12-16 s).

Table 4. Clinical data in dogs

Nr case	Age	Breed	Sex	CRT (Sec)	Mucous Memb.	CF.	RF.	Temp. (°C)	A FAST	T FAST	Haemorrhages			Peristaltism	Abdominal tenderness	Respiratory disturbance
											Int.	Ext.	Site			
1	2 years	Half breed	F	2	congested	-	-	38,3	-	-	-	-	-	√	√	-
3	5 years	Bichon	F	2	pink	-	-	-	-	-	-	-	-	√	√	-
6	2 years	Labrador	F	3	congested	-	-	39,5 ↑	-	-	-		-	-	-	-
8	6 years	Half breed	F	1	congested	128	48 ↑	39,5 ↑	(-)	-	-	-	-	-	√	-
9	2 years	Half breed	F	-	pink	120	70 ↑	39,1	-	-	-	-	-	-	-	-
10	4 years	Labrador	F	-	pale pink	88	31	37,7 ↓	(-)	-	√	-	petechiae: lip and vulva		X	-
11	3 years	Mioritic	F	2	pale pink	-	-	38,3	-	-	-	-	-	-	-	-
12	3 years	BuldFran	M	2	pale	163 ↑	53 ↑	38,5	-	-	-	-	-	-	-	wet rales,cough
14	6 months	Half breed	M	5	pale	-	-	39,5 ↑	-	-	√	-	oral cavity	-	-	-
15	3 months	Beagle	M	3	pale pink	104 ↓	-	37,2	-	-	-	-	-	-	-	-
16	2 years	Half breed	F	1	pale pink	82 ↓	43 ↑	39	(+)	(+)	-	-	-	-	√	whooping cough
17	8 years	Pinscher	F	-	-	-	-	38,6	-	-	√	-	hematemesis	√	√	reacted gastric mucosa
18	4 years	Half breed	M	2	pink	-	-	38,5	(-)	(-)	-	-	-	-	X	-
19	4 years	Tosa Inu	F	-	-	-	-	36,7 ↓	(-)	(+)	√		hemotorax	-	-	-
20	2 years	Husky	F	2	-	-	-	-	(-)	(-)	-	-	-	-	-	-
21	8 years	Husky	F	3	pale pink	-	-	38,7	-	-	-	-	-	-	-	-
22	2 years	Half breed	F	2	pale pink	120	24	40,6 ↑	(-)	-	-	√	Muco-haemorrhagic sialorrhea	-	-	-
24	5 months	Beagle	M	1	congested	110 ↓	56 ↑	38,9	-	-	-	√	oral cavity	-	-	-
25	5 years	Vizsla	M	-	pale pink	130	-	39,4 ↑	-	-	-	-	-	-	√	-
26	8 months	Half breed	M	2	pink	160 ↑	62 ↑	38	(-)	(+)	√	-	hemopericard	-	√	sever dispneea, pulmonary edemas
27	2 years	Dalmația	M	2	pale pink	130 ↑	40 ↑	39	(-)	(-)	-	-	-	-	√	-
28	1,5 years	Germ Shep	M	-	congested	-	-	40,9 ↑	-	-	-	-	-	-	-	slightly enlarged kidney
29	6 years	Bichon	F	1	pale pink	-	-	39,1	(-)	-	-	-	-	√	√	edematous gastric mucosa
32	8 years	Labrador	M	3	pale	120 ↑	70 ↑	38,2	(-)	(+)	√	-	hemotorax		√	whooping cough, pulmonary edema
34	2 years	Akita Inu	M	4	pale	110 ↑	30	39,8 ↑	-	-	-	-	-	-	-	-
35	3 months	Germ Shep	M	2	pink	-	-	38,5	-	-	-	-	-	-	-	-
36	3 years	Germ Shep	M	1	pale pink	104 ↑	28	39,1	-	-	-	-	-	-	-	-
37	11,5 years	Bichon	F	2	pink	128	100 ↑	38,7	(-)	(-)	-	√	petechiae	-	-	-
38	6 years	Gold Retri	F	1	pale	120 ↑	116 ↑	39,9 ↑	-	(+)	-	-	-	-	-	dispneea

Table 5. Haematology in dogs

Nr. case	WBC 10^9/L	RBC 10^{12}	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	PLT 10^9/L
Norm	6-17	5,5-8,5	12-18	37-55	60-77	19,5-24,5	31-34	200-500
6	-	-	9,3 ↓	33 ↓	-	-	-	-
8	10,04	7,3	16,5	44,78	61	22,7	36,9 ↑	-
9	23,05 ↑	6,67	14,6	43,33	65	21,9	33,7	194 ↓
11	-	-	16,2	46	-	-	-	-
12	9,2	4,35 ↓	10,7 ↓	36,64 ↓	84 ↑	24,7 ↑	29,3 ↓	63 ↓
14	-	-	17,8	52	-	-	-	-
15	7,67	5,94	12,3	37,89	64	20,7	32,4	376
16	-	-	15,1	41	-	-	-	-
17	14,02	10,02 ↑	20,08 ↑	58,71 ↑	59 ↓	20,7	35,4 ↑	333
18	9,72	6,8	16,1	44,69	66	23,7	36,1 ↑	272
19	10,94	4,91 ↓	11,27 ↓	30,53 ↓	67	22,8	33,5	129 ↓
20	10,95	6,55	15,2	44,89	69	23,2	33,8	234
22	-	-	15,5	44	-	-	-	-
24	-	-	14,6	43	-	-	-	-
25	11,8	8,5	20,2 ↑	59,26 ↑	69,7	23,7	34,1 ↑	289
26	-	-	9,35 ↓	26 ↓	-	-	-	-
27	-	-	18,2 ↑	51	-	-	-	-
28	18,8	8,425	18,9 ↑	53	65	22,95	35,9 ↑	94 ↓
29	5,12 ↓	6,6	13,56	40	67	22,9	34,3 ↑	795 ↑
	7,92	5,81	14,7	36 ↓	62	25,3	40,9 ↑	-
30	-	-	15,6	48	-	-	-	-
32	19,72 ↑	3,67 ↓	9,2 ↓	26,22 ↓	67,9	25 ↑	36,7 ↑	114 ↓
33	13,385	2,38 ↓	5,55 ↓	14,91 ↓	68,3	25,6 ↑	37,45 ↑	59 ↓
34	-	-	8,3 ↓	26 ↓	-	-	-	-
36	-	-	17,7	45	-	-	-	-
38	21,09 ↑	3,77 ↓	9,85 ↓	28,49 ↓	61	22,6	37,1 ↑	338

Table 6. Biochemistry in dogs

Nr. case	BUN mg/dL	Creat. mg/dL	Glu mg/dL	Na mmol/L	K mmol/L	Total bilirubină mg/dL	Acidul lactic mmol/L
Normal	8-28	0,5-1,7	76-119	142-152	3,9-5,1	0,1-1,2	<2,5
6	7 ↓	0,9	97	144,9	4,14	12,1 ↑	
8	9	1,3	122 ↑	146,9	3,86 ↓	0,6	2,3
11	20	1,2	-	146,6	3,85 ↓	1,6 ↑	3,6 ↑
14	13	0,3 ↓	135 ↑	138,8 ↓	3,43 ↓	0,7	0,9
15	14	0,7	130 ↑	143,4	4,47	2,2 ↑	1,1
16	9,6	0,4 ↓	240 ↑	136 ↓	3,79 ↓	0,7	6,3 ↑
17	10	1,2	142 ↑	150,1	3,74 ↓	0,5	2,6 ↑
19	40 ↑	0,7	350 ↑	130,6 ↓	3,21 ↓	-	9,2 ↑
22	13	0,8	110	145,3	3,93	0,5	4,1 ↑
24	21	1,2	149 ↑	146,3	3,98	0,7	1,8
26	12,2	0,5	133,5 ↑	138,6 ↓	4,21	3,7 ↑	2,8 ↑
27	9	1,2	128 ↑	141,8 ↓	3,87 ↓	0,7	2,3
28	16,5	1,1	121,66 ↑	147,5	4,35	4,5 ↑	3,65 ↑
29	12	1,1	123,33 ↑	144,2	4,04	3,5 ↑	2,36
30	29,5 ↑	2 ↑	97,5	149,6	3,9	1,8 ↑	1,5
32	34,5 ↑	2,8 ↑	260 ↑	125,5 ↓	5,16 ↑	-	15,5 ↑
34	12	1	-	142,7	3,82	5,2 ↑	1,9
36	14	1	131 ↑	146,1	3,97	0,6	2,2
37	19	1,2	76	148,1	4,6	-	3,7 ↑
38	-	0,5	150 ↑	137,9 ↓	4,32	0,5	2,7 ↑

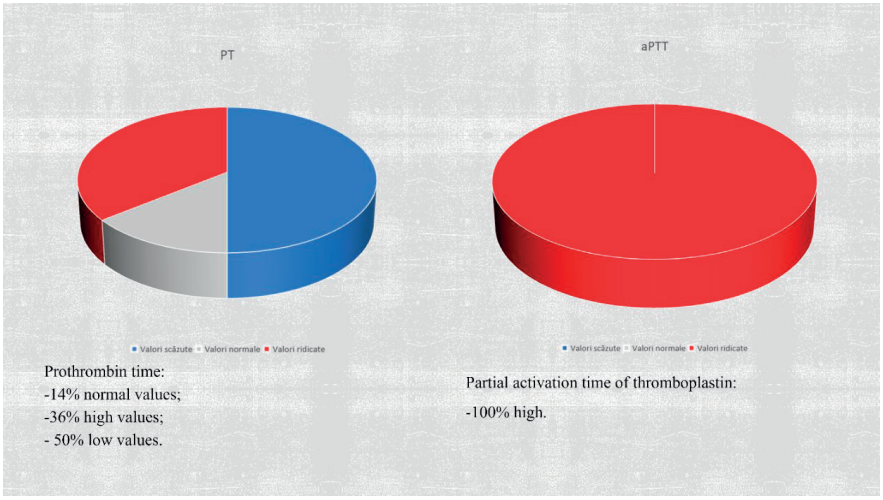

Figure 1. Coagulogramma in dogs

CONCLUSIONS

In conclusion, out of 40 cases, 4 of them a cat and 3 dogs suffered simultaneously from kidney and liver diseases and if we take them individually, we had 2 cats and 3 dogs with kidney impairment and 2 cats and 8 dogs with liver disorders. Because we collected the data from the archive of the Emergency Veterinarian Hospital, our cases were accidental/intentional poisonings, we do not know the exact ingested dose, nor the time passed between the intoxication and the clinical examination, because of that we should take into account that the clinical signs, the laboratory data and paraclinical results can slightly differ depending on this and the individual response of the animal (breed, age, sex).

The recommendation for veterinarians is to make all possible examination of the patient as complete as possible, history, clinical examination, laboratory investigations and other paraclinical examinations show be done (echography, radiography) and prevention should be observed regarding to the protection of animals against this type of intoxication (Valchev et al., 2006).

A practicing clinician if at the clinical examination observe pale, anaemic mucous membranes, increases of the capillary refill time and external or internal bleeding, anticoagulant rodenticide poisoning should be put among the differential diagnose list.

At the haematology part thrombocytopenia and low haemoglobin and haematocrit would be concerning, yet coagulogramma is the most trustful tulle to diagnose anticoagulant rodenticide intoxication, where we can see that the prothrombin time will increase and the partial activation thromboplastin time increase will be helpful in putting a positive diagnose.

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THE CURATIVE EFFICIENCY OF THE CMP-3 IN AVIAN CLOSTRIDIOSIS

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Abstract

The aim of the work was to study the curative-prophylactic efficiency of the complex biologically active microbial preparation and its effect on some growth and development indicators of chickens. The preparation was included in a proportion of 40 g/kg feed, in the composition of the daily ration of the chickens in the experimental group, starting from the first day of life. At the age of 17 days, an infection with Clostridium perfringens was registered in the chickens from both groups, which was treated with the Bromex preparation. As a result of the daily administration in the ration of the complex biologically active microbial preparation, the viability of 100% of the chickens in the experimental group was ensured compared to 72% in the control group; decreasing the titer of pathogenic bacteria E. coli, Enterococcus spp., Clostridium spp. by 6.5-10.6%, in the gastrointestinal tract; increasing the titer of beneficial microorganisms Bifidobacterium spp., Bacillus spp. and yeast fungi by 3.6-15.4%; the increase in the body weight of the chickens at the end of the experiment by 25.5% compared to the control.

Key words*: chicken, complex microbial preparation, avian clostridiosis.*

INTRODUCTION

Avian clostridiosis (anaerobic enterotoxemia, necrotic enteritis) is an acute anaerobic infection caused by *Clostridium perfringens*, types A, C and D (Hutu et al., 2006; Parent, 2008).

C. perfringens is part of the obligate microflora of the large intestine and can be isolated from both sick and healthy birds (Cooper & Songer, 2009; Karim & Khaled, 2013). The bacteria are excreted by the birds in droppings and can be isolated from the walls and ventilators of the bird shelter, from workers ' boots, from feed and litter (Greenham et al., 1987; Craven, 2000; 2001; Parent, 2008; Hafez, 2011).

For many decades, avian clostridiosis was considered a sporadic disease with a minor economic impact.

Currently, it is one of the emerging diseases that threaten poultry farms, due to low productivity, increased mortality, as well as the risk of contamination of products of avian origin intended for human consumption (Hafez, 2011).

The disease occurs in chickens older than 15 days, more frequently in those increased on the ground. The evolution of the disease is favored by nutritional factors (undernutrition, overnutrition), various components of the ration, such as fish meal, wheat or barley flour, high cellulose content or change in the fodder ration; environmental factors (humidity, cold, excessive heat); some diseases with a digestive localization (some bacterioses, viruses or parasitoses) or changing chemotherapeutic preparations (Hutu et al., 2006; Parent, 2008).

Over several decades the monitoring of avian clostridiosis and, in generally, bacterial diseases in poultry youth was carried out by using antimicrobials in water or feed (Dibner & Richards, 2005). But, the intensive use of antibacterial preparations have increased the resistance of pathogens to them (Parent, 2008).

Contemporary approaches to the treatment of avian clostridiosis tend to limit the use of antibiotics in poultry farming and a complete ban on some antibacterial preparations. For these reasons, the identification of some phytogenic and microbial additives, organic

acids, probiotics, prebiotics etc. with immunomudalatory and antibacterial activity (Caly et al., 2015), which could completely replace the use of antibiotics for the control of *C. perfringens* and associated diseases remains paramount for specialists in the field (Thanissery et al., 2010; Agunos et al., 2017; Blake et al., 2021).

The purpose of the work was to study the efficiency of the complex biologically active microbial preparation on growth and development indicators of chickens and curative-prophylactic efficiency in preventing and combating some pathologies of bacterial origin that frequently affect chickens in the first weeks of life.

MATERIALS AND METHODS

As objects of research served: 50 chickens of the breed Lohmann brown 1 day old and the complex microbial preparation (CMP-3).

CMP-3, is a natural microbial complex containing biologically active compounds, derived from *Spirulina (Arthrospira) platensis* biomass and *Saccharomyces* yeast biomass, is a combination of three biologically active extracts, two cyanobacterial: lipid extract (LE), peptidoglycan extract (PPGE) and a mannoprotein extract RSM-MP obtained from yeast biomass from sediments from the production of red *Merlot* wine (Beșliu et al., 2022).

LE and PPGE are obtained in the same technological flow with two other products from dry cyanobacterial biomass, residual after obtaining some peptide extracts. LE is obtained by extraction with 96% ethyl alcohol, biomass: extractant ratio 1:2 v/v, at a temperature of 60˚C for 1 hour with periodic stirring from the remaining biomass after the extraction of pigments.

PPGE represents the insoluble sediment, remaining after the extraction of pigments, LE and proteoglycan extract, which is dried in an oven at a temperature of 50 ± 5˚C to a constant mass with 7-8% moisture and ground to powder.

CMP-3 is obtained by gradually imbibing of LE and RSM-MP in PPGE and drying at 50 ± 5˚C for 24-48 hours. CMP-3 is a dark green powder with 97.47 ± 0.12% d.w. and moisture of 2.53 ± 0.12%, containing not less than 800 mg/g PPGE, 100 mg/g RSM-MP, 70 mg/g LE. Due to its complex composition, CMP-3 contains a wide spectrum of biologically active substances - amino acids and proteins, lipids, including phospholipids and diglycerides, polysaccharides, including mannoproteins and sulfated polysaccharides, pigments, especially anthocyanins and β-carotene, macro-, microelements and possesses a total antioxidant activity of 35.2 ± 0.9% inhibition (ABTS) and of the antioxidant enzymes catalase (CAT) of 826.91 ± 3.04 mmol/min./mg protein and SOD of 71.66 ± 1.64 U/mg protein.

RSM-MP, which is a part of CMP-3, possesses significant antioxidant properties, CAT (catalase) and SOD (superoxid dismutase) activities and nutritional value, which is evidenced by the content of essential and immunoactive amino acids, the presence of macro-, microelements and anthocyanins (Besliu et al., 2022; Chiselita et al., 2023).

Microalgae and cyanobacteria represent a natural source rich in compounds with biological activity which could be used as functional ingredients (Maddiboyina B. et al., 2023). Their valuable biochemical composition, high quality proteins, balanced profiles of fatty acids, vitamins, antioxidants, minerals and their useful properties can be applied in the formulation of new products (Spolaore et al., 2006).

The chickens selected for the experiment had the average veight 36.10 ± 2.00 g, body development and were free clinicall healty.

During the research, the following technological parameters were respected: density of placement of chickens at 1m^2 of surface; light and temperature regime according to age; nutrition regime according to age; water at discretion.

The experiment took place under the conditions of the laboratory Methods of Disease Control and Prophylaxis of the Scientific and Practical Institute of Biotechnologies in Zootechny and Veterinary Medicine of the Republic of Moldova.

The scheme of the experiment is presented in Table 1.

Table 1. Scheme of the experiment

Specification	Control batch	Experimental batch
Biological material	1 day old chickens	1 day old chickens
Number of chickens	25	25
Duration of the experiment, days	28	
CMP-3 inclusion dose, %	-	4

The chickens were fed with combined fodder balanced in proteins, vitamins, minerals and amino acids in accordance with age requirements.

In the composition of the combined feed, intended for feeding chickens from the experimental batch, in the basic feed, 4% of the quantity of fodder yeast was substituted by the same amount of CMP-3 (1 kg basic feed: 40 g CMP-3).

The feeds were administered to the chickens of the corresponding batches, daily for 28 days.

The composition of the feeds used in the experiment and their nutritional value is shown in Table 2.

Table 2. Composition (%) of the combined feed used in the experiment and its nutritional value

Components, %	Combined feed	
	Control batch	Experimental batch
Maize	54	54
Soy meal	22	22
Wheat	13	13
Feed yeast	5	1
Sunflower meal	3	3
CMP-3	0	4
Premix	2	2
Fodder calcium	1	1
Metabolic energy, kcal/kg feed	2703	2698
Crude protein, %	22.1	21.9

The clinical condition of the chickens from both groups was monitored daily by assessing the general condition, behavior, consumption of food and water, consistency of fecal masses, appearance of plumage and cloacal region.

Bacteriological study of feces and sensitivity of *C. perfringens* to antimicrobian preparations was performed according to the usual methods (Licker et al., 2019; Carp-Cărare et al, 2014).

For microbiological investigations were used: culture media such as Nutrient Agar, HiCrome *E. coli* Agar, HiCrome Bacillus Agar Base, HiCrome Endo Agar, HiCrome Kligler Iron Agar, HiCrome Streptococcus Lactis Differential Agar, Anaerobic Agar, MRS Agar, HiCrome Candida Differential Agar, HiCrome Sabouraud Dextrose Agar; putties for staining smears according to the Gram method etc.

The dynamics of the body mass of the chickens from both groups was assessed by individual weighing.

The therapeutic efficacy of CMP-3 on *C. perfringens* was established based on the clinical status of the chickens, bacteriological results of the droppings, recovery dynamics and weight gain.

RESULTS AND DISCUSSIONS

In poultry factories, for several decades, the administration of antibacterial preparations has been practiced, for the prophylaxis of potential disease outbreaks (Starciuc et al., 2018), due to the intensive breeding system limited space for exploration of chickens, closed sheds etc. (Kumar & Patyal, 2020).

In the experiment carried out, in the first days of the chickens' life, no antimicrobial preparations were administered because the goal was to determine the curative-prophylactic efficiency of the CMP-3 preparation in the case of the evolution of bacterial pathologies (colibacillosis, salmonellosis, clostridosis etc.) specific for this age period.

Initially, at the age of one day, the chickens from both groups weighed 36.10 ± 2.00 g, were apathetic, consumed little feed and water. This condition was determined by transport stress. Already the next day the condition of the chickens improved.

In the age period 1-16 days, a positive dynamic of weight gain was observed in the chickens from the experimental batch, favored by the consumption of the combined feed with the addition of CMP-3. Thus, on the 15th day of the experiment, the body weight of the chickens in the experimental batch exceeded that of the control batch by 18.21%.

At the age of 17 days, an infection with *C. perfringens* was registered in the chickens from both groups. To determine the path of infection of the chickens with the pathogen, the water used in their daily consumption, the feed and the dust in the room were examined bacteriologically. The cause of infection was determined to be compound feed containing 3.2×10^7 CFU/g *C. perfringens*.

For these reasons, both contaminated feeds were excluded from the chicks' feed and substituted with microbiologically qualitative equivalent feeds, which were then administered to the corresponding groups throughout the duration of the experiment.

Clarke (2007) mentions that trypsin inhibitors commonly found in soybean meal provide an ideal environment for the development of *C. perfringens.* Timbermont et al. (2011) described the risk of developing necrotic enteritis in chickens fed cereals with a high digestible fiber content, such as oats, barley, rye and wheat. At the same time, the incidence of necrotic enteritis in chickens fed with protein-rich rations is ten times more frequent compared to those fed corn-based rations (Cooper & Songer, 2009).

In the food rations used in the experiment, soybean meal constituted 22%, being a valuable source of protein and energy necessary for the growth and development of chickens. Thus, there is a high probability that this component favored the increase in the amount of the pathogen in the combined feed used in our experiment.

According to the results of the specialists in the field, the prevention of avian necrotic enteritis consists in controlling the risk factors, one of which is the protein-rich ration (Parent, 2008; Hafez, 2011).

Another predisposing factor of avian clostridiosis is invasion by coccidia (Avi et al., 2023; Rodgers et al., 2015; Timbermont et al., 2011). In young birds, coccidiosis frequently precedes cases of clostridiosis (Timbermont et al., 2011).

In the experiment carried out, the invasion with coccidia was not observed, neither in the chickens from the control batch, nor from the experimental one. The extent of invasion with *Eimeria* spp. oocysts was 8% in both batches, and the intensity of invasion was 0-2 oocysts in the field of view of the microscope.

In the control batch, clinically, the disease manifested itself with diarrhea, dehydration, greenish-white feces with a fetid odor, dyspnea, drowsiness, ataxic gait. The same clinical signs in chickens are described by Cooper et al. (2013).

On the 3rd day of manifestation of signs of disease were detected 4 corpses, and on the 4th-5th day another 3 corpses. Morbidity was 100%, mortality - 28 %. Thus, 7 chickens died in the control batch, and the remaining ones lost significantly in weight compared to the chickens in the experimental batch.

According to Uzal et al. (2014), *C. perfringens* strains can produce up to 17 different toxins, and necrotic enteritis induced by *C. perfringens* in chickens leads to sudden death, with mortality rates of up to 50% (Lee et al., 2011).

In the experimental batch, clinical, the disease manifested itself with apathy, 9 chickens presented inappetence and diarrhea. Morbidity constituted 36 %, mortality - 0%.

Hutu et al. (2006) describes avian clostridiosis in chickens over 15 days old, with very rapid evolution (1-5 days), or chronic (7-30 days), with the following symptoms: inappetence, drowsiness, cyanotic crest, hemorrhagic diarrhea, or feces frothy, and in chronic forms, diarrhea with greenish-white feces and fetid smell.

Hafez (2011) specifies that infections with *C. perfringens* in poultry can cause several clinical manifestations and lesions include necrotic enteritis, necrotic dermatitis, cholangiohepatitis as well as gizzard erosion. However, subclinical infection can take place too.

At the necropsy examination of some corpses, catarrhal-hemorrhagic enteritis was found, and in others, intestines with thickened walls and gas bubbles. No pathological changes were observed in the internal organs, although some authors describe *C. perfringens* is also associated with fibrosing hepatitis (Craven, 2000).

Hutu et al. (2006) mentions that at the necropsy examination, the main lesions are found in the anterior portion of the intestine, which presents catarrhal-hemorrhagic and necrotic enteritis; dilated intestine; ulcers covered with fibrin; peritonitis.

Clostridiosis of chickens from both groups was treated with bromex preparation containing 200 mg/mL enrofloxacin and 15 mg/mL bromhexine HCl. Enrofloxacin is a chemotherapeutic with a broad spectrum of antimicrobial action, part of the last generation of synthetic quinolones, with a notable bactericidal activity on Gram positive and Gram negative bacteria. Compared to the rest

of the quinolone group, enrofloxacin has less toxicity.

C. perfringens showed a sensitivity zone to bromex of 26 mm, to enrofloxacin of 20 mm and to amoxicillin of 18 mm.

Bromex was administered in water, 0.3 ml per litre, for 5 consecutive days.

The clinical condition of the chickens in the experimental batch improved on the 3rd day of treatment, and those in the control batch on the 7th day. The chickens became active, began to consume the feed, droppings became consistent, their color normalized.

Chickens in the experimental batch went through the infection more easily, survived completely and the recovery period was faster.

In the control batch the recovery of the chickens was uneven and more later.

As a result of the study of the microbiological composition of the droppings of the chickens from both groups, an increase in the titer of the total number of germs (NTG) by 0.3 log CFU/g (3.5%), the titer of the beneficial microorganisms *Bifidobacterium* spp. ($P<0.001$), *Bacillus* spp. ($P<0.05$) and yeast fungi was recorded by 0.3-1.5 log CFU/g and the decrease by 0.6-1.0 log CFU/g of the titer of pathogenic and conditionally pathogenic microorganisms *E. coli* ($P<0.001$), *Enterococcus* spp. ($P<0.05$), *Clostridium* spp., in the gastrointestinal tract of chickens from the experimental group, compared to the control group (Table 3).

Table 3. Titer of strains of microorganisms in chicken droppings from the control and experimental batch at the end of the experiment, log UFC/g

Indicators	Control batch	Experimental batch
NTG	9.8±0.04	10.1±0.05
E. coli	9.5±0.04	8.5±0.07***
Enterococcus spp.	9.3±0.23	8.3±0.05*
Clostridium spp.	9.3±0.30	8.7±0.02
Lactobacillus spp.	9.8±0.15	9.6±0.07
Bifidobacterium spp.	9.2±0.04	10.7±0.07***
Bacillus spp.	9.5±0.07	9.8±0.04*
Fungi	9.4±0.06	9.9±0.02**

Note: * - $P<0.05$; ** - $P<0.01$;*** - $P<0.001$

The titer of beneficial microorganisms in the gastrointestinal tract of chickens from the experimental batch increased by 3.6-15.4% compared to the control, and that of pathogenic and conditionally pathogenic ones decreased by 6.5-10.6% (Figure 1).

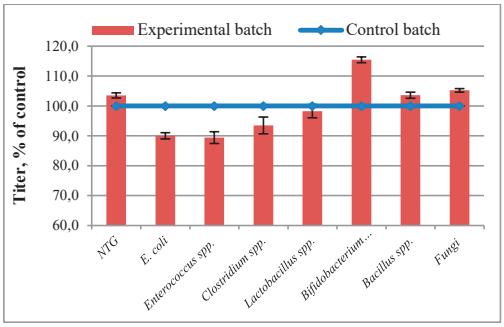

Figure 1. The effect of experimental feed with CMP-3 on the titer of different microorganisms in the gastrointestinal tract of chickens

The results of this study are in agreement with those reported by Craven (2000), who observed a reduction in the number of *C. perfringens* in the intestinal contents of chickens and proved that the numbers of *C. perfringens* detected in the fecal droppings of birds generally reflected the numbers found in the ileum.

In recent research conducted on rats, Li et al. (2019) demonstrated that *S. platensis* benefits health by inhibiting chronic inflammation and intestinal dysbiosis. Modulation of intestinal permeability occurs due to high molecular weight lipids and polysaccharides in *S. platensis*. The lipids have been shown to contain high levels of polyunsaturated fatty acids, including linolenic acid which is a precursor of arachidonic acid and several kinds of sterols (Bensehaila et al., 2015). High molecular weight polysaccharides with multiple biological activities, including anti-cancer, anti-oxidant, immunomodulatory, hypolipidemic and hypoglycemic, anti-thrombotic, anti-viral, regulation of the gut microbiota properties, and other biological activities (Kwak et al., 2015; Xiaopeng et al. 2023; Yadavalli et al., 2023).

One of the main conditions for obtaining maximum productivity from chickens raised in an industrial system is to ensure adequate, balanced feed, in which the main role is played by energy value and protein content (Starciuc et al., 2018).

The energy and protein content of the combined feeds administered to the chickens in the control batch was, respectively, 2703 kcal/kg and 22.1%, and in the experimental batch, respectively, 2698 kcal/kg and 21.9% (Table 2). So, the nutritional value of the feed

was sufficient to ensure the physiological needs of the chickens.

During the experiment chickens from the control batch consumed 9.69% less feed (Table 4) compared to those from the experimental batch, because during the period of infection with *C. perfringens* they showed inappetence, which negatively influenced not only feed consumption, but also the weight gain.

Table 4. Combined feed consumption, g/day

Period, weeks	Control batch	Experimental batch
1	14.86	16.33
2	14.51	19.71
3	21.72	20.40
4	36.85	40.00
Average	21.98	24.11

It is important to note that CMP-3, added in the amount of 4% in the chickens ration, did not negatively influence the smell and taste of the feed, but significantly favored the weight gain of the chickens.

The chickens from the experimental batch at 28 days had a body mass of 238.40 g, weight corresponding to the age, and those from the control batch registered only 190.00 g, which is 13.64% less than the minimum norm and 25.47% ($P<0.001$) less compared to the chickens from the experimental group (Table 5).

Table 5. Body mass of the chickens during the experiment, g

Age, days	Control batch	Experimental batch
8	58.70±0.11	58.84±0.35
15	62.62±0.10	74.02±0.04***
28	190.00±6.18	238.40±14.20***

Note: ***- P<0.001

It is known that in birds that have been ill with clostridiosis, the intestinal villi remain atrophied, and this subsequently affects productivity and feed conversion (Avi et al., 2023). Because of this, the chickens in the control group at the age of 28 days had a body mass below the age limit.

The results obtained by us indicate that the administration of CMP-3 in the daily ration of chickens, in a proportion of 4%, had a beneficial effect on the body by stimulating the beneficial microflora in the gastrointestinal tract of chickens and inhibiting the pathogenic and conditionally pathogenic one, strengthening the immune system, the chickens suffering a milder form of the disease, which allowed the mortality of the chickens to be reduced to 0% and the faster recovery after the disease, by returning to the normal body weight, characteristic of the age.

Analogous results were obtained by Hashim et al. (2018), Santovito et al. (2019), Chiselita et al. (2023) who established the effectiveness of different mannoprotein preparations from yeasts, in maintaining performance, health of chickens and reducing mortality, caused by *C. perfringens* infections.

Finally, we can mention that thanks to its antioxidant activity and its natural and complex composition, which combines biologically active substances of cyanobacterial and yeast nature, CMP-3 strengthens the immune system of young poultry, improves the health of the intestine by stimulating the growth of beneficial bacteria and inhibiting the development of pathogens, including clostridia, increasing the resistance of the chickens' body, which allows to reduce the negative impact of the infection. At the same time, the post-infection recovery of the chickens is faster, as evidenced by their weight gain.

CONCLUSIONS

The use of CMP-3 in the feed of chickens ensured the survival of all chickens in the experimental batch, compared to 72% in the control group; decreasing the titer of pathogenic bacteria *E. coli*, *Enterococcus* spp., *Clostridium* spp. by 0.6-1.0 log CFU/g, increasing the titer of beneficial microorganisms *Bifidobacterium* spp., *Bacillus* spp. and yeast fungi by 0.3-1.5 log CFU/g, compared to the control in the gastrointestinal tract of chickens; the increase in the body weight of the chickens from the experimental batch at the end of the experiment by 25.47% ($P<0.001$) compared to the chickens from the control batch.

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ALFAXALONE SEDATION FOR CENTRAL VENOUS CATHETERIZATION IN A DOG UNDERGOING HEMODIALYSIS: CASE REPORT

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Abstract

A 5 years old Shar Pei female, diagnosed with acute kidney injury was referred for hemodialysis therapy. The dog was presented with lethargy, lack of appetite, severe weight loss, dehydration (7-10%) and pale mucous membranes. To prevent exacerbation of preexisting comorbidities, in order to sedate a renal patient, a safe anesthesia protocol requires understanding the kidney disease pathology and hemodialysis therapy implications. A central venous catheter (CVC) was placed under a light sedation with additional oxygen therapy. Sedation was induced intravenously with alfaxalone (Alfaxan® multidose), on a peripheral catheter. The dose was titrated until full relaxation of the patient was observed. During the procedure, there were no major hemodynamics changes in the patient. Alfaxalone is a short-acting and rapid duration anesthetic with minimal or no cardiovascular consequences. When given titrated to effect, it represents the best choice for short sedation in central venous catheterization for acute renal patients undergoing hemodialysis.

Key words*: alfaxalone, sedation, CVC, hemodialysis, kidney.*

INTRODUCTION

Sedation is a daily routine in clinical practice, used for minor medical procedures or in premedication prior to general anesthesia, imaging studies or to manage fractious or excited animals. Because of their availability and efficacy, intramuscular (IM) administration of ketamine, opioids, α2-adrenoceptor agonists or their combinations has been widely used for sedation or general anesthesia in dogs (Tamura et al., 2015).

Alfaxalone is a synthetic neuroactive steroid that produces good quality sedation with fast onset, good muscle relaxation, minimal cardiovascular effects and mild respiratory depression when administered intravenously at clinically doses in dogs, making it a clinically acceptable induction agent for unstable canine patients. In dogs, alfaxalone has been demonstrated to have less cardiorespiratory side effects and minimal to none cardiovascular changes when it is used at clinical doses. These hemodynamic changes are dose-dependent. Overdoses can cause increased heart rate, hypotension and hypoventilation (Psatha et al., 2011). Intravenous administration of alfaxalone produces a smooth induction of anesthesia and rapid recovery with dose-dependent cardiorespiratory depression (Kato et al., 2021).

Acute kidney injury (AKI) is characterized by sudden renal parenchymal injury, and is associated with decreased renal function, retention of uremic waste products, fluid, electrolyte and acid-base imbalances. Short-term prognosis of AKI is affected by multiple factors including the etiology (which influences the reversibility of the injury), comorbidities, complications and treatment options (Rimer et al., 2022).

Despite advances in management of AKI and the increased availability of renal replacement therapies, the overall case fatality rate remains high both for dogs managed medically or with hemodialysis. Despite comprehensive diagnostic workup, in a substantial percent of patients with AKI the etiology is unknown at presentation (Rimer et al., 2022).

Central venous catheter (CVC) use is increasingly higher in veterinary practice in the management of critical patients when peripheral venous access is difficult or even

impossible to achieve, and is the primary element in the therapy of patients with acute kidney injury or chronic kidney disease undergoing hemodialysis (HD). Vascular access is the most important basic requirement of successful extracorporeal renal replacement therapy. Hemodialysis is a therapeutic procedure that use the extracorporeal circulation of a patient's blood to improve azotemia, fluid overload, electrolyte and acid-base abnormalities characteristic of the uremic syndrome (Ștefănescu & Viţălaru, 2018).

The aim of this study was to evaluate the dose-dependent sedative and anesthetic effects of IV alfaxalone administered for central venous catheterization in a dog undergoing hemodialysis.

MATERIALS AND METHODS

A 5 years old female Shar Pei, 18 kg body weight, diagnosed with acute kidney injury was referred for hemodialysis therapy. The dog was presented with lethargy, appetite loss, severely weight loss, dehydration (7-10%) and pale mucous membranes. Results from a complete blood cell count (CBC), biochemistry, and urine analysis submitted at that time were abnormal.

Hemodialysis, hydro-electrolytic rebalancing and partial parenteral nutrition were the main parts of the complex therapy. The placement and installation of the central venous catheter is a key factor for the therapeutic success of hemodialysis.

The American Society of Anesthesiologists (ASA) physical status classification system in this study was performed based on physical examination of the dog at presentation and hematology and biochemistry results, imaging diagnostic tests and underlying disease.

Monitored parameters before sedation, as a part of pre-anesthetic evaluation, were: heart rate (HR, beats/minute), peripheral oxygen saturation (SpO_2), respiratory rate (RR, breaths/minute), electrocardiogram (ECG) with 3-leads and rectal temperature (TºC). The blood pressure (BP) was measured using High-Definition Oscillometry (HDO), with the dog placed in sternal recumbency. The systolic (SAP, mmHg), diastolic (DAP, mmHg) and MAP (mean arterial pressure) were monitored.

The successful clipping and placing of an peripheral IV catheter was recorded as part of the sedation assessment.

The patient did not receive any premedication, the sedation protocol being initiated with a 3.2 mg/kg IV bolus of alfaxalone. The assigned drug was injected at a rate of 25% of the total calculated dose (0.8 mg/kg) every 15 seconds over the first 60 seconds. The patient was directly induced by alfaxalone bolus administration, followed by CRI at a rate of 0.18 mg/kg/min using an infusion syringe pump for delivery. During sedation the patient received fluid therapy with Ringer (rate and dosage: 10 ml/kg/h) given for hydro-electrolytic rebalancing.

During the CVC placement procedure, vital parameters were measured using a patient monitoring system and additionally assessed with a stethoscope and by observing thoracic movements. Heart rate was measured by thoracic auscultation and lead II-ECG wave. Respiratory rate was obtained by direct observation of thoracic movements. SpO_2 and HR variability were obtained simultaneously using non-invasive pulse oximetric recording. SAP, DAP and MAP were measured on the forelimb using the HDO method, with an appropriately sized cuff placed above the carpus (width 40% of limb circumference - C1 cuff). Rectal temperature was measured with a digital thermometer.

Due to mild respiratory depression (hypoventilation) during the procedure oxygen supplementation via face mask (low-flow oxygen system) was started. The patient was maintained during the CVC placement procedure with CRI of alfaxalone and oxygen. Oxygen supplementation was stopped when the patient no longer presented hypoventilation and SpO_2 returned to physiological parameters.

The anesthetic and cardiorespiratory effects were evaluated before administration and sequentially at every 5 minutes, after the IV administration of alfaxalone until the recovery of the patient. The procedure lasted 20 minutes. All this time the patient was under sedation protocol and any abnormal movements, ECG alteration or other abnormal measurements were noted, recorded and monitored. Recovery from sedation was uneventful.

RESULTS AND DISCUSSIONS

The aim of this study was to evaluate the clinical efficacy and cardio-respiratory effects of alfaxalone as an induction agent for sedation protocol in dogs with severe systemic diseases such as acute kidney injury prior to hemodialysis.

At the admission, the patient had elevated blood biochemistry results on renal interest parameters BUN: 210 (RR: 7-21 mg/dL), CRE: 18.9 (RR: 0.4-1.4 mg/dL). Moderate hyperkalemia appears on the electrolyte imbalance side K^+: 6.8 (RR: 3.4-5.6 mmol/L), low value of CA: 12.6 (RR: 8.6-11.8 mg/dL) and value of PHOS: 18.5 (RR: 2.9-6.6 mg/dL), ALB: 2.0 (RR: 2.6-3.5 g/dL). Results from complete blood cell count (CBC) revealed: HGB: 3.2 (RR: 12-18 g/dL), HCT: 12.76 (RR: 37-55%), MCHC: 28.8 (RR: 31-39g/dL) and RDWc: 12.1 (RR: 14-20%) consistent with anemia. Urinalysis was carried out from urine obtained through ultrasound guided cystocentesis and showed a UPC ratio of >=0.5 to <2.0 (proteinuric), pH of 5.1, microalbumin >=25 mg/L. Based on these results and IRIS AKI Grading criteria, the patient is staged in grade V. Using ASA Physical Status Classification System, the patient was assigned as IV risk class, based on clinical signs and blood work results (a patient with severe systemic disease that is a constant threat to life – unstable renal disease with high uremia).

Prior to sedation, the patient had SAP between 184 to 195 mmHg, DAP 93 to 98 mmHg and MAP between 123 and 130, using HDO. The cardio-respiratory parameters, were: HR with 110 to 113 beats/minute, SpO_2 within 97 to 98%, RR within 25 to 28 breaths/minute and rectal temperature of 37.2ºC. Respiratory sinus arrhythmia was expressed on lead II-ECG, with no other electrical conductivity changes.

The stage of sedation that made the patient relaxed and positioned in lateral recumbency for the CVC procedure was observed 2 minutes after CRI administration of alfaxalone. There were no significant changes in ECG - sinus rhythm and blood pressure SAP: 167 to 173 mmHg, DAP: 80 to 90 mmHg and MAP: 109 and 117. HR decreased slightly to 99-101 beats/minute, RR from 28 to 12 breaths/minute and SpO2 between 96 and 97%.

After starting the CRI administration of alfaxalone, the patient expressed mild respiratory depression. Hypoventilation lasted about two minutes, SpO_2 dropped between 94 and 95% and RR decreased from 12 to 5 breaths/minute. Oxygen supplementation was mandatory and the patient received it via face mask at a flow rate of 5 L/min until the peripheral oxygen saturation was above 97%. It is cited that alfaxalone can cause short-lived hypoxia and oxygen supplementation was applied to the patient (Rodríguez et al., 2012).

Intravenous administration of alfaxalone was able to provide a dose-dependent anesthetic effect and good to excellent short-term sedation when administered as CRI. The CRI dose of 0.18 mg/kg/minute after a bolus dose of 3.2 mg/kg, produced adequate sedation without causing severe cardiorespiratory depression but a clinically acceptable decrease in vital parameters such as HR, RR and BP. Transient respiratory depression was noted and managed on time for the medical procedure to be performed safely for the patient, with no clinical implications.

In the present study, the dog exhibited mild muscular tremors and paddling during the early period of recovery from IV alfaxalone sedation.

CONCLUSIONS

The pharmacokinetic and pharmacodynamic properties of alfaxalone including its rapid onset and short duration of action with high total body clearance, resulted in little accumulation following IV administration.

The bolus dose of alfaxalone required for sedation and adequate neuro-depressive effect was 3.2 mg/kg IV and a total dose of 3.6 mg/kg (0.18 mg/kg/minute) administered in CRI for 20 minutes of procedure for central venous catheterization, provides clinically useful and effective sedation without causing severe cardiorespiratory depression, but a mild respiratory distress with no clinical implications was noted for this patient. An important factor that must be considered in patients with grade V AKI undergoing sedation for the CVC procedure, is that the clinical status may have a negative impact on the effects of anesthetic agents on homeostasis.

When it is used as a sedation agent in compromised dogs, alfaxalone seems to have a suitable cardiovascular pharmacodynamic profile with a mild respiratory side effect, that can be managed with thorough monitorization.

In conclusion, a dose-dependent anesthetic effect and smooth recovery were obtained with IV administration of alfaxalone in bolus following titration of the dose in a CRI on a patient that was not premedicated.

This protocol provided adequate neuro-depressive effect and effective sedation causing mild and transient respiratory distress with no severe cardiac depression or other clinical implications, for central venous catheterization.

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HYDRONEPHROSIS, DIABETES AND FELINE UROLOGIC SYNDROME IN A MIXED BREED TOMCAT: CASE REPORT

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Abstract

An 8 years old intact tomcat, was referred with urinary incontinence and polydipsia. After ultrasound examination, the patient was diagnosed with feline urologic syndrome and hydronephrosis. Acute urethral obstruction is the result of a physical or functional obstruction and can be life-threatening due to azotemia, hyperphosphatemia and hyperkalemia. Following the onset of obstruction, regardless of its nature, bilateral hydronephrosis can occur. Biochemistry revealed low serum sodium, serum albumin (ALB), alanine aminotransferase (ALT), blood urea nitrogen (BUN), total proteins (TP) and glucose (GLU) were elevated. On further examination, by determining serum fructosamine concentration, stress hyperglycemia was excluded, and diabetes mellitus was diagnosed. This article brings valuable insights into the complex pathology of feline urologic syndrome, paving the way for enhanced clinical strategies and potential preventive measures.

Key words*: hydronephrosis, diabetes mellitus, feline urologic syndrome, serum fructosamine, hyperglycemia.*

INTRODUCTION

Feline urologic syndrome is a clinical syndrome in cats that encompasses the following typical signs of lower urinary tract disease such as hematuria, periuria, dysuria or anuria, straining to urinate and urethral obstruction with or without polysystemic sign of urethral obstruction (depression, anorexia, vomiting, dehydration, hypothermia) (Willeberg, 1984; Osborne et al., 1984).

Nowadays, the common term used as a synonym for feline urologic syndrome is lower urinary tract disease which also encompasses various disorders with heterogenous causes that can affect the urinary bladder and/or urethra of cats. Feline lower urinary tract disease can be classified into urolithiasis, neoplasia, bacterial urinary tract infection, anatomic malformations, feline idiopathic cystitis and iatrogenic (behavioral, metabolic, neurological). Etiological studies from 1996 found evidence of urethral plugs in 60%, urethral calculi in 20%, stricture or neoplasia with <5% combined, and the rest did not have clear evidence of physical obstruction. In a more recent study, which was carried out in 2008, the incidence of feline idiopathic obstruction was found to be higher than 53%, with 29% urolithiasis, and only 18% urethral plugs. Feline urethral obstruction is associated with 90-95% survival rate, with reported recurrence rates of 15-40% (Cooper, 2015).

The ethiopathogenesis of feline idiopathic cystitis may involve interactions between three main components: environmental factors, neuroendocrine system and urinary bladder of the affected cats (He et al., 2022).

Urethral obstruction can be the result of physical obstruction (due to urolithiasis and formation of struvites, urates, calcium oxalate crystals; mucous plugs) or a functional obstruction (such as idiopathic obstruction) (Bartges et al., 2015).

Urinary tract infection (UTI) refers to the adherence, multiplication and persistence of an infectious agent within the urogenital system that causes an associated inflammatory response and clinical signs. Many felines have asymptomatic or subclinical bacteriuria. UTIs can be classified as complicated (anatomic or functional abnormalities of the urinary tract, comorbidities, recurrent infection or treatment failure, multiple episodes within one year) and uncomplicated (normal urinary tract anatomy

and function). Lower UTI have signs that are non-specific and can be seen in any disease of the lower urinary tract (Dorsch et al., 2019).
Hydronephrosis can be unilateral or bilateral and results from partial or complete obstruction of the ureters. Obstruction of urine flow causes a progressive dilation of the renal pelvis. As bilateral hydronephrosis presents with acute azotemia, rapid diagnosis and urgent therapy are required if renal function is to be re-established (Elliot et al., 2017).
Feline diabetes is one of the most common endocrinopathies in cats. Most affected cats are older than 8 years old, with a peak of incidence between 10 and 13 years. Protracted hyperglycemia and glucosuria cand lead to the classic clinical signs of polyuria, polydipsia, polyphagia and weight loss. If left untreated or inadequately controlled, ketoacidosis can occur. It is important to differentiate patients with clinical diabetes mellitus from those with transient hyperglycemia or mildly increased blood glucose (BG). In felines, when BG concentration exceeds 250-300 mg/dL, glycosuria will typically develop (Behrend et al., 2018).
Marked hyperglycemia in cats is shown in acute stress associated with blood sampling, and therefore this will not ensure a reliable result. Fructosamine is a glycated serum protein (albumin and other plasma proteins) that reflect glycemic control over the previous 2 to 3 weeks. Its concentration directly depends on the plasma glucose concentration (Lutz et al., 1995; Kaneko et al., 1992; Crenshaw et al., 1996).
Felines with transitory hyperglycemia and diabetes mellitus cand be differentiated based on fructosamine concentrations. Fructosamine is a valuable parameter in the diagnosis and metabolic control of diabetes mellitus in cats (Reusch et al., 1993).

MATERIALS AND METHODS

An 8 years old, intact tomcat with a body weight of 4.8 kilograms was referred for a second opinion nephrology consult on 13th of September, 2023. Owner reported that the patient presented one episode of urinary incontinence the previous day and is polydipsic. The patient did not present any signs of feline urologic syndrome (FUS) such as: stranguria, dysuria, lethargy, anorexia, vocalization and excessive grooming of the perineum. Patient did not express any inappetence or lack of appetite. The patient usually consumed dry and wet food. During clinical examination, the following findings were noted: body temperature of 38.6°C, 5% dehydration, dry mucous membranes and painful sensitivity and defensive reaction to deep palpation of the abdomen.
The complete blood count (CBC) was performed on Vetscan HM5 Hematology (5-part Differential) and determined: white blood cells (WBC), lymphocytes (LYM), monocytes (MON), neutrophils (NEU), eosinophils (EOS), basophils (BAS), LYM%, MON%, NEU%, EOS%, BAS%, red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width-coefficient of variation (RDWc), red blood cell distribution width-standard deviation (RDWs), platelet (PLT), mean platelet volume (MPV), plateletcrit (PCT), platelet distribution width-coefficient of variation (PDWc) and platelet distribution width-standard deviation (PDWs).
Serum biochemistry was performed on Vetscan VS2 Chemistry Analyzer with a comprehensive diagnostic panel that determined: albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), amylase (AMY), total bilirubin (TBIL), blood urea nitrogen (BUN), calcium (CA), phosphorus (PHOS), creatinine (CRE), glucose (GLU), sodium (NA^+), potassium (K^+), total proteins (TP) and globulin (GLOB).
Urinalysis was performed on Vetscan UA Urine Analyzer and determined leukocytes, ketones, nitrites, urobilinogen, bilirubin, glucose, protein, specific gravity, pH, blood, ascorbic acid, microalbumin, calcium, creatinine and protein/creatinine.
Abdominal ultrasound with a major focus on the kidneys is essential in the diagnosis and management of kidney-related diseases. The kidneys are easily examined in longitudinal and transverse scan planes, and most pathological changes in the kidneys are distinguishable by ultrasound. The exam was initiated in the

longitudinal scan plane, parallel to the long diameter of the kidney (Hansen et al., 2015).

Decompressive cystocentesis, prior to urethral catheterization, was done to lower intraluminal bladder pressure in order to facilitate retropulsion and improve catheterization. No complications emerged following this procedure.

Ultrasound guided cystocentesis was performed using a 21-gauge needle attached to a 10 milliliters syringe, which was inserted percutaneously at a 45 degrees angle. The needle was attached to an IV extension tubing, three-way stopcock and a 20 milliliters syringe.

Urethral catheterization was performed using an open-end tomcat catheter (3.5 Fr, 14 cm) and retrograde urohydropropulsion. After decompression, a closed-end urinary catheter was placed. The urinary catheter was maintained in place for the next 48 hours of hospitalization.

The sedation protocol included alfaxalone 3 mg/kg IM with ketamine with a dose of 2 mg/kg IV and midazolam with a dose of 0.3 mg/kg IV. Lidocaine gel was applied topically to the glans penis of the patient in order to achieve a topical anesthesia.

Rehydration and electrolyte rebalancing were initiated by continuous rate of infusion (CRI) with Ringer solution (rate and dosage 7 ml/kg/h) and partial parenteral nutrition based on levo-amino acids was administered (rate and dosage: 0.6-0.8 g amino acids/kg/24 h). The active ingredients in the levo-amino acids solution are: L-isoleucine, L-leucine, L-lysine monoacetate, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, L-valine, L-arginine, L-histidine, L-alanine, N-acetyl-L-cysteine, L-cysteine, Glycine, L-proline, L-serine, L-tyrosine, N-glycine-L-tyrosine dehydrate, N-glycine/L-tyrosine. It contains a total of 100g/L amino acids and Enteric dialysis supplements based on *Streptococcus thermophiles, Lactobacillus acidophilus, Bifidobacterium longum* and *Lactobacillus casei*, nutritional supplements based on amino acids combined with a peptide that supports kidney functions and supplements that help maintain and support the urinary tract (based on DL-methionine) were introduced as adjuvants in the therapy.

The initial approach in managing diabetes mellitus was to initiate the administration of insulin glargine at a starting dose of 0.5 units (U) per kilogram every 12 hours. Blood glucose was monitored hourly for the first 48 hours of hospitalization. For the next 72 hours of hospitalization BG was determined every 3-4 hours. The patient was given a low carbohydrate diet.

RESULTS AND DISCUSSIONS

On the 13th of September 2023, the patient had the following serum biochemistry modifications: Na^+ 118 mmol/L (RR = 142-164 mmol/L), ALB 4.7 g/dL (RR = 2.2-4.4 g/dL), ALT 121 U/L (RR = 20-100 U/L), BUN 41 mg/dL (RR = 10-30 mg/dL), TP 8.4 g/dL (RR = 5.4-8.2 g/dL) and GLU 627 mg/dL (RR = 70-150 mg/dL). There were no changes regarding the CBC. Urine sediment did not present any crystals. Urinalysis was carried out from urine obtained through ultrasound guided cystocentesis and showed leukocytes +3500 cell/uL, glucose +328 mmol/L with a specific gravity of 1.020 and a pH of 5.0. A urine culture sample was submitted to a microbiology laboratory and the result was negative (0 colony-forming unit - CFU).

Following the elevated result of the BG, quantification of serum fructosamine concentration was performed and the following result was obtained: 772.60 µmol/L (RR = 190-365 µmol/L). Abdominal ultrasound was performed and the following changes were observed: over distension of the urinary bladder with moderate parietal reaction with no corpuscular elements detected with tendency of sedimentation. The left kidney with a diameter of 5.83/3.3 centimeters had a regular contour with a slightly globular appearance, suitable cortico-medullar ratio and hydronephrosis of grade 1-2 without urolithiasis. The right kidney had a diameter of 5.3/3.2 centimeters and revealed the same aspects. Hydronephrosis is typically graded visually and can be divided into five categories going from a slight expansion of the renal pelvis to end-stage hydronephrosis with cortical thinning (Hansen et al., 2015).

Medical management of urethral obstruction consists of maintaining adequate renal

perfusion, reversing life threatening electrolyte disturbances, minimizing visceral pain and alleviating the urethral obstruction (Hall et al., 2015).

By performing retrograde urohydropropulsion, the patient was catheterized and 395 milliliters of yellow colored urine were eliminated. Urinary bladder lavage was performed twice daily with NaCl 0.9% (Tsuruta et al., 2022).

The patient was hospitalized and submitted to intravenous fluid therapy for electrolyte rebalancing and partial parenteral nutrition based on levo-amino acids (rate and dosage: 0.6-0.8 g amino acids/kg/24 h).

Alfaxalone provided induction of anesthesia with stable respiratory and cardiovascular effect (Warne et al, 2015).

Midazolam produces reliable muscle relaxation and is often administered with other anesthetic drugs that do not provide sufficient muscle relaxation alone (Riviere et al. 2018).

Muscle tone is reduced because of the effects on the dorsal horn of the spinal cord (Smith et al., 2009).

Ketamine is a rapid acting general anesthetic that also has significant analgesic activity and a lack of cardiopulmonary depressant effects. Effects on muscle tone are described as being variable, but ketamine generally either causes no changes in muscle tone or increases tone (Plumb, 2018). The patient was preoxygenated and oxygen was delivered by mask. During the urethral catheterization, the patient was continuously monitored (body temperature, blood pressure [BP], pulse oximetry - SpO_2). The following values were noted: body temperature 38.2-38.5°C, mean arterial BP 83-95 mmHg and SpO_2 95-97%. The patient did not present any complications following this protocol (Grubb et al., 2020).

The use of an indwelling urinary catheter for the measurement of urine output allowed for a personalized patient fluid therapy. During the first night of hospitalization, rehydration was established by CRI with Ringer solution (rate and dosage 5 ml/kg/h) and urinary output was 10 ml/kg/h. For the next 24 hours, urinary output was approximately 9.53-9.64 ml/kg/h with a CRI of 7 ml/kg/h during the day and 5 ml/kg/h over the night. After 48 hours, the indwelling urinary catheter was removed. Urethral obstruction did not reoccur (Cosford et al., 2020; Hall et al., 2015).

Blood glucose curves were initiated and maintained over a period of five days and monitored hourly in the first 48 hours (Figures 1 and 2). In the following days of hospitalization, the BG levels were monitored every 3-4 hours until the day of discharge from the clinic (Figures 3-5) (Rand et al., 2005).

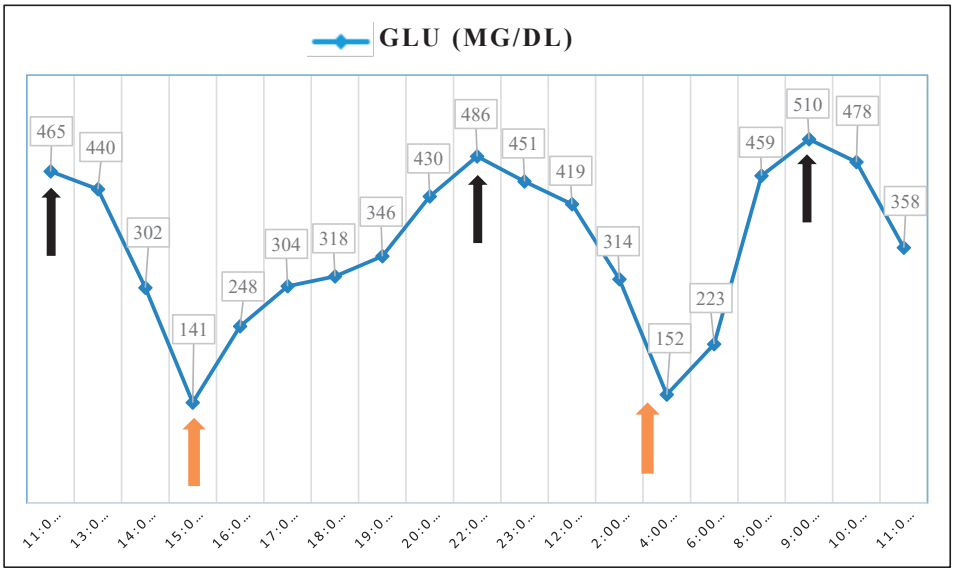

Figure 1. Evolution of GLU in the first 24 hours of hospitalization (original)
Black arrows - administration of insulin glargine, orange arrows - food administration

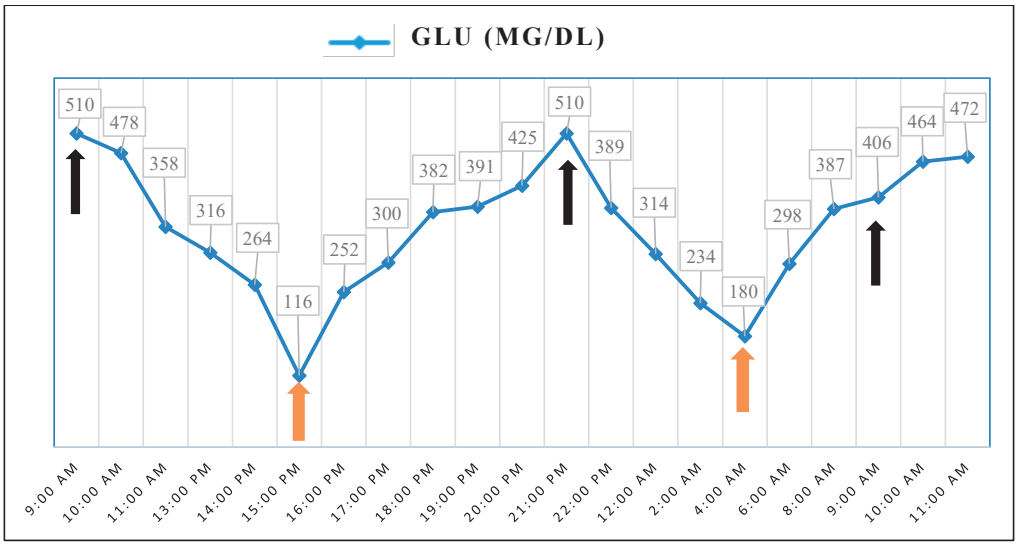

Figure 2. Evolution of GLU in the next 24 hours of hospitalization (original)
Black arrows - administration of insulin glargine, orange arrows - food administration

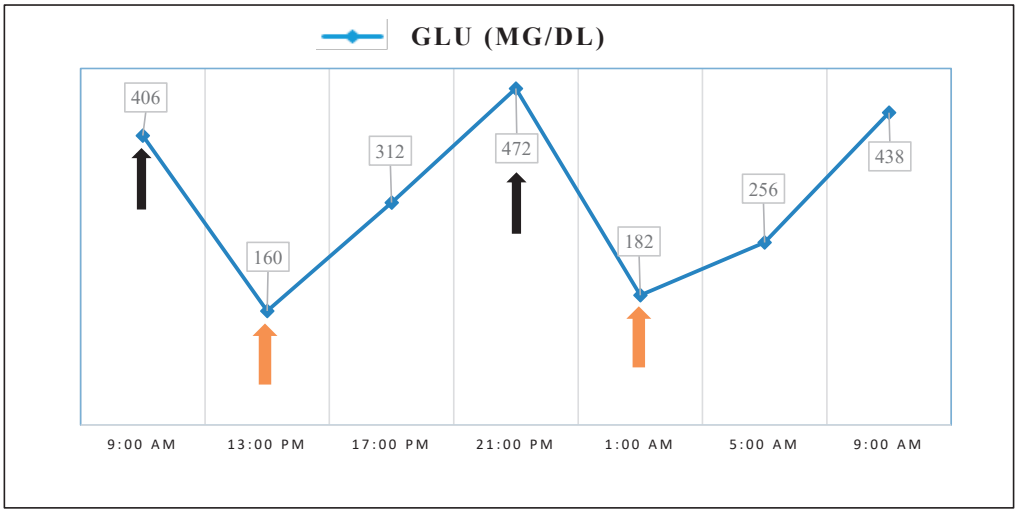

Figure 3. Evolution of GLU on the third day of hospitalization (original)
Black arrows - administration of insulin glargine, orange arrows - food administration

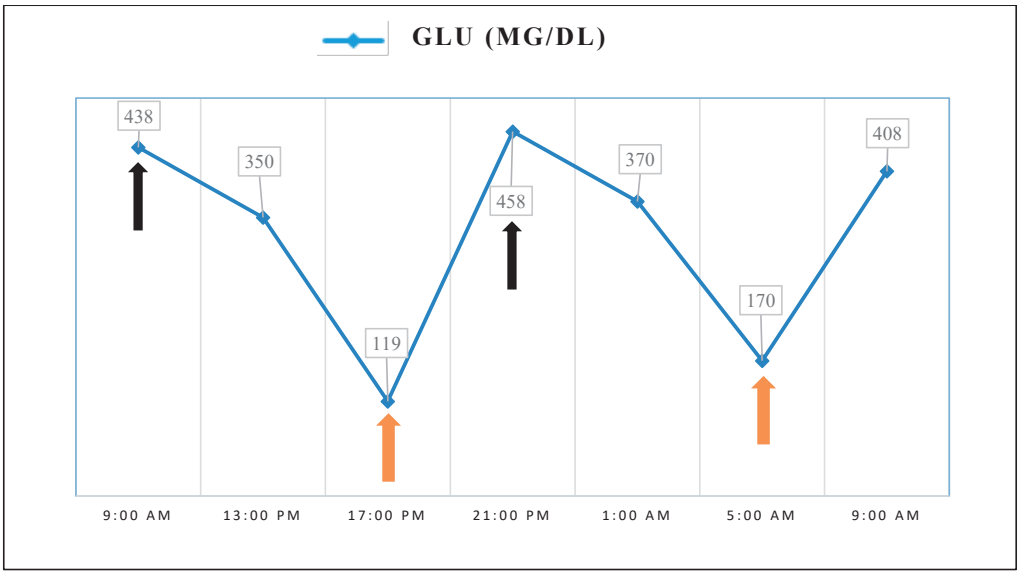

Figure 4. Evolution of GLU on the fourth day of hospitalization (original)
Black arrows - administration of insulin glargine, orange arrows - food administration

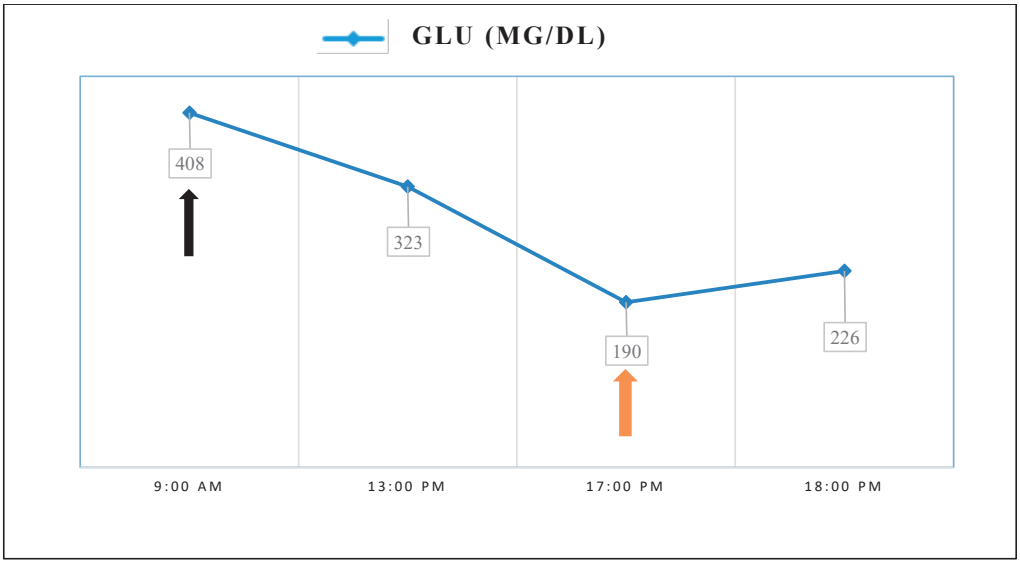

Figure 5. Evolution of GLU on the final day of hospitalization (original)
Black arrow - administration of insulin glargine, orange arrow - food administration

On discharge day, serum biochemistry values were in normal ranges, except BG, which was elevated (226 mg/dL; RR = 70-150 mg/dL). Serum fructosamine concentration was 412.1 µmol/L (RR = 190-365 µmol/L). The patient was discharged with the following recommendations:

- determination of BG before food administration at 9:00AM; if BG is >400 mg/dl, insulin glargine will be administered with a dose of 0.5 UI/kg;
- insulin glargine will be administered every 12 hours (9:00 AM and 21:00 PM);
- BG will be tested at 9:00 AM, 17:00 PM and 21:00 PM;
- food will be administered at 9:30 AM, 17:30 PM and 21:30 PM;
- nutritional supplements based on amino acids combined with a peptide (300 mg every 12 hours) and supplements that help maintain and support the urinary tract (based on DL-methionine - 250 mg TID);
- low carbohydrate diet.

Five days after discharge a CBC, serum biochemistry, urinalysis, abdominal ultrasound and serum fructosamine were reassessed. The owner was informed to administer the dose of insulin the day of the reexamination. There were no abnormalities on the CBC. Serum biochemistry revealed a mild elevation of blood glucose 190 mg/dL (RR = 70-150 mg/dL) with a serum fructosamine concentration of 382.5 µmol/L (RR = 190-365 µmol/L). The urine sample was obtained by ultrasound guided cystocentesis and urinalysis showed a decrease in leukocytes, glucose +105 mmol/L with a specific gravity of 1.029 and a pH of 6.0. Abdominal ultrasound showed an improvement of the morphology of the kidneys.

There was no sign of obstructive uropathy (hydronephrosis), except a mild dilation of the renal pelvis.

The evolution of BG and serum fructosamine is presented in Figure 6.

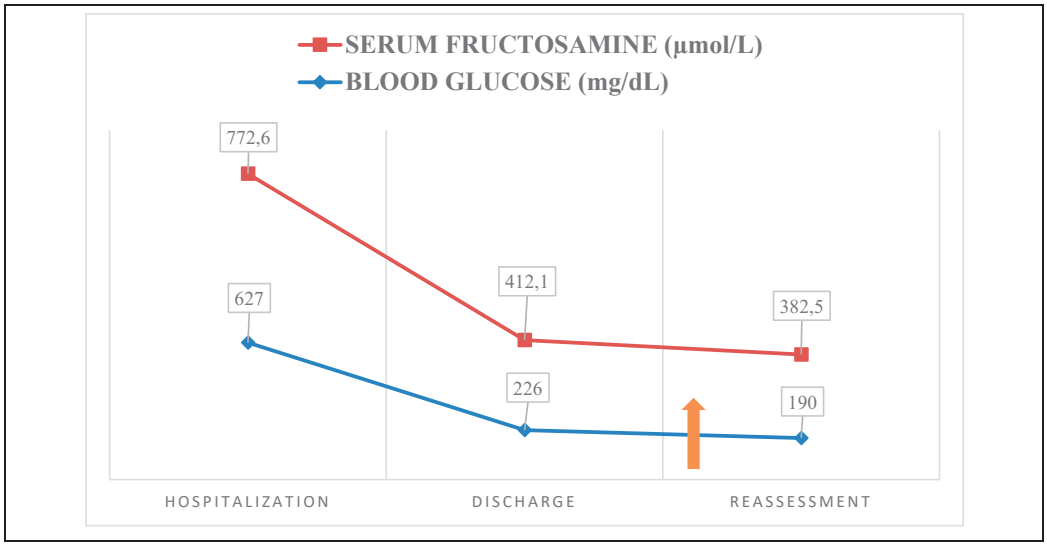

Figure 6. Evolution of BG and serum fructosamine of the patient during hospitalization, at the time of discharge and reassessment

CONCLUSIONS

Serum fructosamine concentration is a valuable parameter for the diagnosis and metabolic control of diabetes mellitus.

Understanding the effects of diabetes and hyperglycemia, acid-base status is paramount to safely anesthetize and sedate diabetic patients.

Alfaxalone provides induction of anesthesia with stable respiratory and cardiovascular effect. Each patient requires an individualized treatment plan with frequent reassessmen and tempering depending on the patient's response.

In this study, the administration of insulin glargine resulted in no reported clinical signs of hypoglycemic episodes.

An accurate management of feline lower urinary tract disease can lead to remission of hydronephrosis and full recovery.

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P-WAVE VARIATIONS IN CENTRAL VENOUS CATHETERIZATION IN A CANINE PATIENT UNDERGOING HEMODIALYSIS: CASE REPORT

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Abstract

A 5 years old, Shar Pei intact female, presented with lethargy, progressive weight loss, pale mucous membrane and severe dehydration (7-10%). The patient was diagnosed with acute on chronic renal disease (ACKD), polycystic kidney disease (PKD) and non-regenerative anemia and was referred for hemodialysis. A central venous catheter was placed under light sedation. During central venous catheterization, the cardiac rhythm was monitored by ECG. A key factor for a favorable outcome of the procedure is to understand the correlation between central venous catheterization and cardiac electrical activity. ECG monitoring goal was to observe alterations in P-wave morphology, which reflects atrial depolarization, during central venous catheterization. By monitoring the P-wave variations with extensive care is possible to observe the valuable cardiovascular dynamics in the context of central venous catheterization, aiding clinicians in optimizing patient care and safety of the procedure.

Key words*: electrocardiogram, P-wave, hemodialysis, central venous catheter, kidney.*

INTRODUCTION

The existence of structural or functional alterations in one or both kidneys for a period longer than three months is known as chronic kidney disease (CKD). In dogs, the disease progresses, although the rate at which it does so varies greatly. In dogs, known indicators linked to the course and result of chronic kidney disease (CKD) include anemia, low body condition score, proteinuria, hypertension, and hypoalbuminemia (Polzin, 2011; Bartges, 2012). Numerous factors, such as glomerular diseases, infections, recurrent ischemic episodes, nephrotoxicity, neoplasia, prior acute kidney injury (AKI), or urinary obstruction, have been linked to the pathophysiology of chronic kidney disease (CKD); however, the etiology of these factors is frequently unclear at the time of presentation and stays unclear over the course of the disease (O'Neill et al, 2013; Rudinsky et al, 2018). Acute-on-chronic kidney disease (ACKD), a sudden decline in kidney function, can occur in animals with stable chronic kidney disease (CKD). AKI and ACKD may have similar pathophysiology, clinical presentations, and laboratory abnormalities (Rudinsky et al., 2018).

Central venous catheterization is frequently used for critically ill patients when peripheral catheters aren't adequate due to vascular fragility or thrombophlebitis. They're preferred for administering blood products or medications and for frequent blood sampling, central venous pressure monitoring, or oxygen saturation monitoring Campbell et al., 2012).

Central venous catheters (CVCs), or central lines, vary in size and lumens. For them to be central venous, the catheter tip should be in the cranial or caudal vena cava. In dogs, if it's inserted into the external jugular vein, it's called a central venous jugular catheter (CVJC) (Hughes et al., 2000).

Hemodialysis is a therapeutic procedure that uses the extracorporeal circulation of a patient's blood to improve azotemia, fluid overload, electrolyte and acid-base abnormalities characteristic of the uremic syndrome. Hemodialysis is used for the management of both acute kidney injury and chronic kidney disease that is refractory to conventional medical therapy (Ștefănescu et al., 2018).

The use of non-invasive and invasive monitoring devices can help veterinary practitioners in the detection of organ dysfunction and patient's state of health (Farry et al., 2018).

Electrocardiography (ECG) is a non-invasive, inexpensive, non-hazardous easy to use diagnostic technique that identifies cardiac arrhythmias, cardiac chamber enlargements, myocardial diseases, ischemia, heart failure, conduction defects (heart blocks, bundle branch blocks) (Wess et al., 2010). Electrocardiogram involves electrodes attaching to the skin and it is frequently used to measure heart rate and heart rate variability. Several electrodes are brought into close contact with the skin, and the ECG is measured from the potential difference between the electrodes. The electrodes must be stable and in close contact with the skin (Ohno et al., 2022).

P-wave indexes are markers of atrial conduction derived from the electrocardiogram. P-wave indexes may reflect the accumulation of subclinical atrial pathology including atrial inflammation, fibrosis, and a quantifiable alteration in atrial conduction (Nattel et al., 2008).

P-wave represents atrial depolarization. The impulse starts in the sinoatrial node on the roof of the right atrium and travels downwards and towards the left (Nattel et al., 2008).

These records provide the foundation for case management and are of particular importance in the critical patient whose treatment plans will likely be detailed and complex, and may change frequently. The fragile physiological state and lack of reserve of these patients will result in little tolerance for missed or incorrect treatments (Farry et al., 2018).

MATERIALS AND METHODS

A 5 years old, 15 kg, intact female Shar Pei, diagnosed with acute on chronic kidney disease and polycystic kidney disease was referred for hemodialysis therapy on October 17th, 2023. The dog was presented with the following symptoms: lethargy, appetite loss, weight loss, dehydration (7-10%, considerable loss of skin turgor), body temperature of 37^{o}C and dry mucous membranes. The complete blood count (CBC) was performed on Vetscan HM5 Hematology (5-part Differential) and determined: white blood cells (WBC), lymphocytes (LYM), monocytes (MON), neutrophils (NEU), eosinophils (EOS), basophils (BAS), LYM%, MON%, NEU%, EOS%, BAS%, red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width-coefficient of variation (RDWc), red blood cell distribution width-standard deviation (RDWs), platelet (PLT), mean platelet volume (MPV), plateletcrit (PCT), platelet distribution width-coefficient of variation (PDWc) and platelet distribution width-standard deviation (PDWs).

Serum biochemistry was done on Vetscan VS2 Chemistry Analyzer with a comprehensive diagnostic panel that determined: albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), amylase (AMY), total bilirubin (TBIL), blood urea nitrogen (BUN), calcium (CA), phosphorus (PHOS), creatinine (CRE), glucose (GLU), sodium (NA+), potassium (K+), total proteins (TP) and globulin (GLOB).

Urinalysis was performed on Vetscan UA Urine Analyzer and determined leukocytes, ketones, nitrites, urobilinogen, bilirubin, glucose, protein, specific gravity, pH, blood, ascorbic acid, microalbumin, calcium, creatinine and protein/creatinine.

A comprehensive urinalysis procedure was performed utilizing a urine specimen acquired via ultrasonography-guided cystocentesis. This method involves the precise insertion of a needle through the abdominal wall into the urinary bladder, facilitated by real-time ultrasound imaging guidance, to obtain a sterile urine sample for diagnostic evaluation.

Blood pressure was evaluated with VET BP Doppler with manometer (Figure 1). The Doppler method is a technique used to indirectly measure blood pressure by evaluating the Doppler shift, which refers to the change in frequency of sound waves emitted by blood flow. This method involves using a Doppler device to detect the frequency shift caused by the movement of blood within arteries.

The procedure for ascertaining a patient's blood type encompasses the meticulous identification

of distinct antigens situated on the surface of their erythrocytes, thereby elucidating the precise blood group classification essential for clinical management and transfusion compatibility assessments. For this purpose, it was used a quick test to determine in less than 5 minutes if the patient's blood type is DEA 1.1 positive or negative.

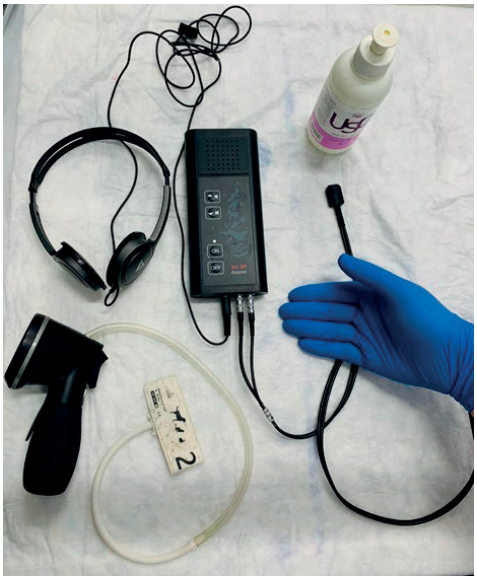

Figure 1. VET BP Doppler kit
used to take the patient's blood pressure

Hemodialysis, hydro-electrolytic rebalancing and partial parenteral nutrition were the main goals of the complex therapy. Rehydration was established by fluid therapy with Ringer continuous rate of infusion (CRI), (rate and dosage: 10 ml/kg/h) for electrolyte rebalancing and partial parenteral nutrition based on levo-amino acids (rate and dosage: 6 ml/kg/24 h, 0.6-0.8 g amino acids/kg/24 h). Enteric dialysis supplements (based on *Streptococcus thermophiles*, *Lactobacillus acidophilus*, *Bifidobacterium longum* and *Lactobacillus casei*), calcium-based phosphorus binders, renal diet and nutritional supplements were introduced as adjuvants in the therapy for supporting kidney functions.

For this patient was chosen Haemocat Signo V 12 Fr (Figure 2). This type of catheter is a temporary double-lumen catheter for extracorporeal blood treatments and specifically designed for use in acute dialysis. Also, this CVC has a length of 20 centimeters and a diameter of 12Fr.

The central venous catheter was placed using the Seldinger method.

Figure 2. Haemocat Signo V 12 FR temporary double-lumen catheter for extracorporeal blood treatments. The central venous catheter used for the patient

The electrocardiogram (ECG) recording and monitoring was performed using Poly-Spectrum 8 Vet Rhythm, 4 clip electrodes with 6-lead ECG (Figure 3).

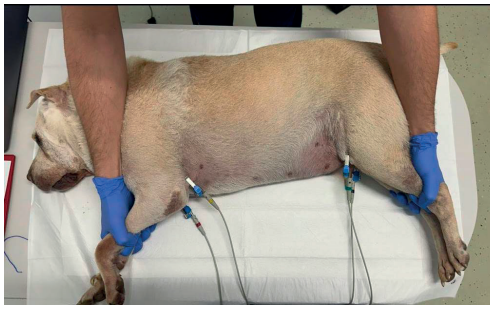

Figure 3. Poly-Spectrum 8 Vet Rhythm, 4 clip electrodes with 6-lead ECG connected to the patient

Electrodes must be positioned as follows: the red one with the inscription RA positioned on the right forelimb, the black one with the inscription RL positioned on the right hind limb, the yellow one with the inscription LA positioned on the left forelimb, the green one with the inscription LL positioned on the left hind limb. The electrodes were placed on the forelimbs and hind limbs, on lateral recumbency in order for the patient to remain calm during

this medical examination, this methodological approach was expected to yield comprehensive and reliable information. The electrocardiogram is used to monitor the patient's vital functions and follow the cardio dynamic changes encountered during procedures of this kind.

RESULTS AND DISCUSSIONS

On October 17th 2023, blood biochemistry revealed: BUN 306 (RR: 7-25 mg/dL), CREA 22.9 (RR: 0.4-1.4 mg/dL), K^+ 6.2 (RR: 3.4-5.6 mmol/L), CA 6.6 (RR: 8.6-11.8 mg/dL), PHOS 24.5 (RR: 2.9-6.6 mg/dL). The patient qualified as grade V acute kidney injury with moderate hyperkalemia and hyperphosphatemia based on subsequent accumulation of metabolic toxins (uremia toxins) and dysregulation of fluid, electrolyte and acid-base balance.

Results from complete blood cell count (CBC) showed HGB 3.7 (RR: 12-18 g/dl), HCT 11.96 (RR: 37-55%), MCHC 30.8 (RR: 31-39g/dl) and RDWc 13.5 (RR: 14-20%) consistent with non-regenerative anemia due to low production of erythropoietin by the kidneys. Urinalysis showed UPC ratio of >=0.5 to <2 (proteinuric), pH of 5, microalbumin >=25 mg/L. Arterial blood pressure was 130-140 mmHg systolic, using Doppler method.

In the first day, 24 hours prior intermittent hemodialysis (IHD) session, the patient was submitted to blood transfusion with 450 ml of blood DEA 1 negative in order to manage anemia (the patient was stable during the blood transfusion, normothermic and normotensive), intravenous fluidotherapy for electrolyte rebalancing and partial parenteral nutrition based on levo-amino acids. Calcium based phosphorus binders, renal diet and nutritional supplements were introduced as adjuvants in the therapy in order to support kidney functions.

The placement and installation of the central venous catheter is a key factor for the therapeutic success of hemodialysis. Selecting the appropriate type and size of central venous catheter is crucial in minimizing the risks associated with this medical procedure.

Hemodialysis was decided as an extracorporeal renal replacement therapy.

Electrocardiogram showed a normal pattern during the cardiologic consult (Figure 4), fact that made it possible for the patient to be subject to the intervention of placing the central venous catheter.

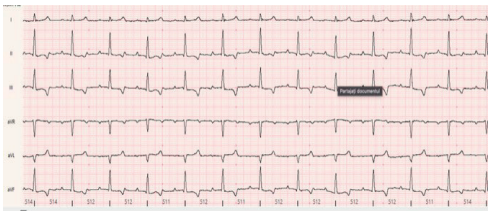

Figure 4. Representation of the patient's electrocardiogram before placement of the central venous catheter

On October 18th 2023 after 24 hours of fluid therapy, a central venous catheter was placed under ~~a~~ light sedation with Alfaxalone and supplemented with oxygen, using aseptic technique (the use of surgical scrub and sterile surgical technique during catheter placement, as well as the use of sterile gloves and the careful handling of catheter line during the procedure).

During the installation of the central venous catheter, it is important to monitor continuously the patient's electrocardiogram in order to observe any changes in the electrodynamics of the heart that may occur during the procedure.

Patients who are afflicted with acute kidney injury often find themselves susceptible to significant shifts in their hydro-electrolytic balance. This intricate balance, governed by the interplay between fluids and electrolytes within the body, frequently manifests in discernible alterations observed on the electrocardiogram. These changes on the electrocardiogram are indicative of the profound impact that the fluctuations in fluid and electrolyte levels can have on cardiac function and rhythm. In this particular case, as depicted in (Figure 4), it was noted that prior to the placement of the central venous catheter, the patient did not exhibit any notable alterations such as cardiac ectopy or arrhythmia, and the electrocardiogram pattern appeared to be within normal parameters.

This type of monitoring helps to ensure the safety of the procedure and well-being of the patient.

At the time of insertion of the central venous catheter, a change in the P-wave was observed on the electrocardiogram (Figure 5).

Figure 5. Representation of the patient's electrocardiogram at the time central venous catheter was inserted

The amplitude of the P-wave changed when the CVC and guide wire were inserted in close proximity of the sinoatrial node through cranial vena cava. At that moment the electrocardiogram registered P-wave elevation arrhythmia in heart electrodynamics. When this arrhythmia occurred, the CVC was withdrawn for approximately 1 centimeter and repositioned slowly. The catheter should be positioned within the vessel lumen. If the catheter enters the right atrium, arrhythmia can be appreciated on the electrocardiogram. Thus, the catheter should be immediately pulled back until the arrhythmia resolves.

Following the withdrawal of the central venous catheter along with the guiding wire, there was a notable observation within a timeframe of approximately 10 seconds: the amplitude of the P-wave exhibited a restorative trend, returning to its baseline level that was evident prior to the initial insertion of the catheter. This phenomenon is visually depicted in (Figure 6), highlighting the dynamic physiological response associated with the removal of the central venous catheter from the close proximity of sinoatrial node.

Figure 6. Representation of the patient's electrocardiogram after the central venous catheter was withdrawn approximately one centimeter

It is very important to confirm the correct position of the CVCs tip due to the mechanical stimulation caused by the final part of the central venous catheter exerted on the sinoatrial node (Jain et al., 2011).

CONCLUSIONS

The variations of the P-wave from the normal aspect of ECG prior the catheterization concluding with an elevated P-wave when the guiding wire and the tip of the catheter reached the close proximity of sinoatrial node remain an incredible marker for practitioners to know when they should retract the catheter and the guiding wire.

The introduction of central venous catheter, a temporary double-lumen catheter, on this case, plays a crucial role in facilitating therapies such as hemodialysis, allowing precise fluid management and electrolyte rebalancing.

Placing a central venous catheter is a procedure that involves risks and it has to be made by a surgeon with experience who knows when to retract the catheter. Monitoring that procedure with an electrocardiograph is mandatory in order to avoid any risk associated with malposition of the central venous catheter.

An accurate ECG diagnosis is an important monitoring tool and it can be used to detect and manage cardiac rhythm abnormalities, during the placing of central venous catheter.

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DYNAMICS OF POST-VACCINATION ANTIBODIES AGAINST CANINE PARVOVIRUS IN DOGS

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Abstract

Canine parvovirosis, or viral haemorrhagic gastroenteritis, is a contagious infectious disease of canids that is characterized by gastrointestinal syndrome and mortality, especially in young puppies. Canine parvovirus type 2 (CPV2) is the pathogen agent of disease, and it is considered antigenically distinct from CPV1, the etiological agent of a disease that causes neonatal mortality in puppies. The high rate of morbidity and mortality in young animals requires the use of specific prophylactic measures. Vaccination against canine parvovirosis is part of the common vaccination scheme; a live-attenuated vaccine is used. The purpose of our study is to evaluate the dynamics of anti-CPV antibodies as a result of vaccination, considering that CPV is extremely important in the sanitary management of dogs.

Key words*: postvaccination antibodies, CPV, dogs.*

INTRODUCTION

Canine parvovirosis, or viral haemorrhagic gastroenteritis, is a contagious infectious disease of canids that is characterized by gastrointestinal syndrome and mortality, especially in young puppies. The etiological agent is Canine parvovirus type 2 (CPV2), which is antigenically distinct from Canine parvovirus type 1 (CPV1), which is the etiological agent of a disease that causes neonatal mortality in puppies (Binn et al., 1970; Carmichel et al., 1994; Parrish, 1999). The origin of CPV2 remains unknown, possibly derived from feline panleukopenia virus (FPV) or wild carnivore FPV-like viruses (Truyen, 1999; Truyen et al., 1998). The first identification of canine parvovirus (CPV) was made by Binn in 1970 (Parrish, 1990), but the first description of illness as a clinical manifestation was made in 1977 in Texas (Burtonboy et al., 1979; Carmichel et al., 1994; Parrish, 1999). After 1977, CPV2 was reported as an emerging etiological agent for severe gastroenteritis in dogs, with epidemic evolution and characterized by decreased appetite, vomiting, mucous/haemorrhagic diarrhoea, and leukopenia (Apple et al., 1979; Burtonboy et al., 1979; Decaro et al., 2005; Kelly, 1978).

The canine parvovirus belongs to the family *Parvoviridae*, subfamily *Parvovirinae*, genus *Protoparvovirus*, and species *Protoparvovirus carnivoran1* (virus name and virus abbreviation are not official ICTV designations). The genome is enclosed in an icosahedral capsid constituted by the combination of two proteins, such as VP1 and VP2, translated from alternative mRNA chains (Martella et al., 2004). Regarding the antigenic structure, CPV presents five antigenic variants: CPV-2a, CPV-2b, the new CPV-2a, the new CPV-2b, and CPV-2c (Singh et al., 2023). Currently, the antigenic variants of the original CPV2 (CPV2a, CPV2b, and CPV2c) are widespread in canine populations around the world. The antigenic and genetic analysis of CPV2 variants isolated in Italy after 2002 highlighted that CPV2c is currently the replacement for CPV2b (Decaro et al., 2005; 2006).

The high resistance of CPV is characterized by its ability to survive at pH variations and, as well, at different temperatures, such as 6 months at 4-10°C, 2 weeks at 37°C, and during the months or years in faeces. It is not inactivated during the action of common disinfectants (e.g., formalin solutions, sodium hypochlorite, and oxidizing agents) and, as well, the action of UV radiation.

Currently, CPV2 affects dogs of all ages. The different variants of CPV2 represent pathogen agents of enteritis and myocarditis in dogs, being characterized by a severe clinical evolution, especially in puppies (Parrish, 1999). The high rate of morbidity (100%) and mortality (90%) is registered in puppies and young dogs between 6 weeks and 6 months of age. The high susceptibility at these ages is a result of decreasing the titre of anti-CPV2 maternal antibodies during the first month. The anti-CPV maternal antibodies start to decrease after 10 days of life (Singh et al., 2023). Due to the severe clinical evolution and the high rate of mortality, it requires the application of general and specific prevention measures. Regarding immunoprophylactic methods, the use of modified live vaccines (monovalent or polyvalent attenuated strains) represents a common practice. The first vaccination of puppies should start between the first 6-8 weeks of age (when the maternal antibody titre is declining), followed by several boosters every 2-4 weeks until the age of 16 weeks or older (Villa Nova et al., 2018). The effectiveness of vaccination depends on the results of both the humoral immune response and the cellular immune response. The duration of postvaccination immunity is related to the degree of memory cell stimulation (Schulz et al., 2010). The decrease in the incidence of CPV infection in the canine population is the result of anti-CPV vaccination.

MATERIALS AND METHODS

In order to assess the dynamics of anti-CPV antibodies as a result of vaccination, a trial was conducted for 12 months postvaccination at 18 adult dogs (different breeds and aged between 1 and 5 years) that have been split into two examination groups, such as **group I (GI)**, which includes 10 females (noted as F1-F10) and **group II (GII)** which includes 8 males (noted as M1-M8). The females in group I were unpregnant during the assessment, and two of them were neutered (F6 and F5).

For this purpose, two-time intervals of blood sampling were established for anti-CPV titre evaluation, during the 12 months after anti-CPV IgG vaccination, as follows:

T1: the first evaluation of anti-CPV IgG titre at 3 months after vaccination.

T2: the second evaluation of anti-CPV titre at 11 months after vaccination.

All dogs included in the trial were dewormed (as a preventive measure), had a good physiological status, and had a complete vaccination programme. All animals, at the time of vaccination as well as at the time of each testing (T1 and T2), were clinically examined, being clinically healthy, without clinic symptomatology of infectious diseases or other diseases. In addition, an evaluation of potential prior exposure to CPV infection has been done with Rapid Immuno-Migration (RIM) Assay for detection of Parvovirus antigen in the faeces of dogs (Witness®CPV, Zoetis, USA). The basic principle of the method is that one anti-CPV-Ab is conjugated with gold particles and another anti-CPV-Ab is fixed on a nitrocellulose membrane. If Ag is present: Ag couples with the conjugate, forming an Ag-Ab complex that will later be captured by Ab fixed on the nitrocellulose membrane with the development of a pink-purple band. All dogs included in the trial have had a negative response at T1 and T2. The last vaccination, before the trail, was carried out 3 months ago, and it has been done with a polyvalent vaccine DHPPI L (modified live strains of Canine Parvovirus 2, Canine Adenovirus type 2, Canine parainfluenza virus, Canine Distemper virus strains, and bacterin of *Leptospira icterohaemoragiae* and *Leptospira canicola*). The vaccination was carried out as part of the annual vaccination scheme for dogs.

In order to assess the anti-CPV postvaccination titre the ImmunoComb® Canine VacciCheck Test Kit (Biogal, Israel) has been used on blood samples. The ImmunoComb®, based on the *dot ELISA* method, is a modified immunoenzymatic test (ELISA), which is used to detect the antibodies and evaluate their titre on whole blood samples or blood serum. According to kit instructions, the measuring of IgG anti-CPV titre is done by a colorimetric interpretation scale (of the kit), and the results are expressed in S units on a scale from 0 to 6. The cut-off S3 represents a value that is equivalent to 1:80 hemagglutinin inhibition units for CPV, which is considered a protective anti-CPV infection. The uppermost spot is the positive control, and it has a distinct purple-grey colour. A colour tone identical to S1 or weaker is considered a negative result. The Comb Scale was used to

evaluate the antibody score. The scale of interpretation that has been done based on the instructions of the manufacturer, has four categories, such as:
S0 and S1: negative humoral response when it sees a poor tone of colour.
S2: inadequate humoral response
S3-S6: positive humoral response (the Ig anti-CPV titer is ≥1:80), when it sees a tone of colour that is equal to or darker than the reference (Biogal, Israel, 2010).

RESULTS AND DISCUSSIONS

The assessment of postvaccination anti-CPV IgG titre for group I (GI) for 12 months recorded the following results expressed both in S units and in hemagglutination inhibit units (HI), included in Table 1.

Table 1. Evolution of postvaccination Ig anti-CPV in group I for 12 months (10 female dogs) according to the Comb scale interpretation

Subjects of GI	Age	Titre of Ig anti-CPV at T1		Titre of Ig anti-CPV at T2	
		S	HI	S	HI
F1	3 years	5	1:320	3	1:80
F2	3 years	6	1:640	4	1:160
F3	2 years	5	1:320	3	1:80
F4	5 years	3	1:80	2	1:40
F5	5 years	3	1:80	2	1:40
F6	3 years	5	1:320	4	1:160
F7	1 year	6	1:640	4	1:160
F8	2 years	4	1:160	3	1:80
F9	4 years	5	1:320	3	1:80
F10	3 years	4	1:160	3	1:80
Titre average (as S)		**4.6**		**3.1**	

The evaluation of the IgG anti-CPV dynamics highlights a progressive reduction during the 11 months post-vaccination for group GI. The post-vaccination titre is maintained at ≥ 3 (1:80 HI units) in 80% of the female dogs tested at time T2. For two of them (F4, F5), an IgG titre was registered below the protection limit at T2 by comparing with the minimum protection value (1:80 SN units / S3). It can be noted that the highest postvaccination anti-CPV IgG titres were recorded at T1, where the average titre value has been 4.6, as S. At T2 time, the value of the average anti-CPV IgG titre was 3.1, as S.
The assessment of postvaccination antiCPV IgG titre for group II (GII) for 12 months recorded the following results expressed both in S units and in hemagglutination inhibit units (HI), included in Table 2.

Table 2. Evolution of postvaccination Ig anti-CPV of group II for 12 months (8 male dogs) according to the Comb scale interpretation

Subjects of GII	Age	Titre of Ig anti-CPV at T1		Titre of Ig anti-CPV at T2	
		S	HI	S	HI
M1	2 years	6	1:640	5	1:320
M2	3years	5	1:320	4	1:160
M3	2 years	6	1:640	5	1:320
M4	5 years	4	1:160	2	1:40
M5	2 years	5	1:320	4	1:160
M6	4 years	4	1:160	2	1:40
M7	1 year	6	1:320	5	1:160
M8	5 years	3	1:80	2	1:40
Titre average (as S)		**3.9**		**2.9**	

The evaluation of the IgG anti-CPV dynamics highlights a progressive reduction during the 11 months post-vaccination for group GII. The post-vaccination titre is maintained at ≥ 3 (1:80 HI units) in 70% of the male dogs tested at time T2. For 3 of them (M4, M6, and M8) an IgG titre was registered below the protection limit at T2 by comparing with the minimum protection value (1:80 SN units/S3). Based on these results, it can be observed that the highest postvaccination antiCPV IgG titres were recorded at T1, where the average titre value was 3.9 (as S), while at T2, the anti-CPV IgG titre was 2.9 (as S).
Based on the results obtained for group I, it can be observed that the postvaccination antiCPV IgG titre is protective for 80% of the subjects during the 11 months (Figure 1). The average titres at T1 and T2 are above the minimum protection level, which suggests that the vaccination was not effective.

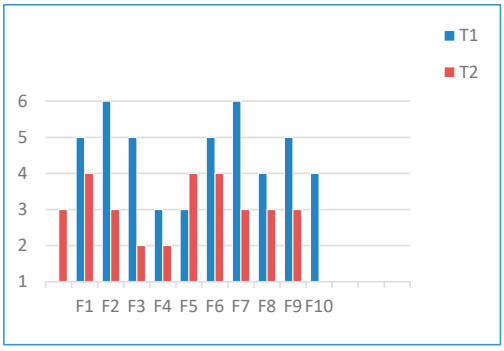

Figure 1. Graphic of S units for Group I

The assessment of the group II results pointed out that postvaccination anti-CPV IgG titre has been maintained at the minimum protective

level for 70% of the subjects during the 11 months. The average values of the anti-CPV IgG titre were at the minimum protection level at T1, respectively, below the minimum protection level at T2, which suggests that the level of protection was adequate against various CPV strains infection for 11 months for 70-80% of dogs (Figure 2.).

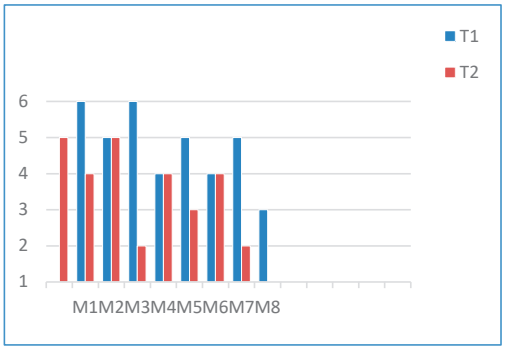

Figure 2. Graphic of S units for Group II

The values of anti-CPV IgG titres recorded at subjects F4 and F5 of GI, and subjects M4, M6, and M8 of GII (below the minimum level) at T2 suggests that the age of vaccinated animals or other exogen factors can influence the immunological reactivity during the 12 months postvaccination. The decrease in titres at the T2 has registered in the dogs aged 4-5 years.

The variations in immunological response are the results of various factors, such as the type of vaccine used, environmental factors, welfare conditions, and the immunocompetence of animal or individual factors (Vila Nova et al., 2018).

One of the important factors that can induce a failure of antiCPV is the high level of maternal antibodies (Nandi et al., 2013), but in our study only adult dogs were included. Other causes could be in relation to veterinary practice, such as storage of vaccine at an inadequate temperature, errors in vaccination, an inadequate programme of vaccination, or failure of the immunogenicity of the vaccine (Decaro et al., 2008; Altman et al., 2017).

CONCLUSIONS

The average values of the anti-CDV IgG titre are maintained above the minimum protection level for 70-80% of dogs, with no significant differences depending on the sex of the animals. The degree of protection against CPV infection is between 70 and 80% during the 11 months after the administration of a modified live vaccine (with CPV-2 strains), which allows us to conclude that during the 12-month period between two sessions of vaccination for canine parvovirus infection an appropriate level of protection is ensured for the majority of tested animals.

The post-vaccination immune response can be influenced by a series of factors, which can occur randomly at any moment of the period considered theoretically to be protective for CPV.

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THE USE OF EXTERNAL FIXATORS FOR THE REPAIR OF SEVERELY COMMINUTED BILATERAL FEMORAL FRACTURES IN A FELINE PATIENT - SINGLE CASE STUDY

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Abstract

Purpose: This article reports the outcome result of a single case study following the use of external fixators to stabilize bilateral comminuted femoral fractures in a cat.
Methods: The full history regarding signalment, radiographic evaluation and postoperative care were included in this article. The patient was regularly monitored with regards to time till onset of weight-bearing, callus formation, joint range of motion, pin site infection and implant reaction, implant stability and loosening-up. The patient obtained good functional recovery with full range of motion.
Conclusions: External skeletal fixators, offer an efficient repair method for severely comminuted femoral fractures in feline patients, obtaining early weight bearing and callus formation, with unimpeded mobility and range of motion.

Key words*: biological fixation, callus, comminuted fracture, diaphyseal femoral fracture, feline external skeletal fixator.*

INTRODUCTION

The use of external fixators in human orthopaedics was first reported in 1897 while a veterinary external fixator was designed by Ehmer in the late 1940s. In its early beginning, this method was associated with frequent complications such as pin tract infection and malunion and lost interest in favor to plates and screws later on (Butterworth, 1993).
Surgical management of diaphyseal fractures in felines can be challenging due to the implant size and strength limitations when approaching smaller feline bones while trying to avoid important neurovascular structures (Zurita et al., 2022).
External fixation has evolved nowadays establishing an appropriate patient specific surgical methodology with optimum pin placement sites for feline limbs for which canine anatomy is often nonapplicable (Prackova et al., 2022).
Usually, external fixation is considered ideal for fracture below the elbow and stifle. Nonetheless when used for above these joints, efficient stabilization may be obtained as well by using an intramedullary pin which will increase resistance to bending forces at the fracture site (Corr, 2012).

MATERIALS AND METHODS

A male feline, European common breed, aged 1 year and weighing 3 kg, was presented for difficulty in using its hind limbs, after being absent from the owners' yard. Owner believed it might have been hit by a car a couple of days prior to admission in the clinic.
On clinical examination, both hindlimbs had pain on palpation, edema, ecchymosis, no open wounds and crepitus on manipulation of the thigh region. The rest of the examination did not reveal other pathologies: TRC<2sec; T=39°C; pale pink moist mucous membranes; mobile, painless palpable lymph nodes; rhythmic heart and vesicular murmur on auscultation; supple abdomen without discomfort on palpation. The patient was bright and alert with unaltered sensation and reflexes.
Biochemical and hematological investigations did not reveal any notable changes (Tables 1 and 2)
Ultrasound and radiological examination of the thorax and abdomen showed no abnormal findings, raising no other concerns.
The radiological examination of the hind limbs revealed the presence of bilateral comminuted diaphyseal femoral fractures and an incomplete

longitudinal fracture involving the proximal femoral fragment of the Right Hind Limb (Figure 1). Such fractures usually result from high velocity trauma so we suspected road traffic accident or high-rise syndrome (Zurita et al., 2022).

Table 1. Biochemistry blood wok

Test	Result	Reference values	Measure unit
ALB	2.2	2.1-3.3	g/dL
TB	<0.06	0.00-0.30	mg/dL
GGT	3	0-5	U/L
ALT	48	0-112	U/L
ALP	90	0-115	U/L
Crea	0.9	1.1-2.0	mg/dL
TC	112.94	70.00-150.00	mg/dL
GLU	137.45	65.00-146.00	mg/dL
Ca	8.66	8.50-10.00	mg/dL
Phos	3.2	3.70-5.60	mg/dL
Bun	15.17	14.00-26.00	mg/dL

Table 2. Haematology blood work

Test	Value	Unit	Reference range
WBC	16.7	$10^3/mm^3$	5.0-11.0
LYM%	11.8	%	0.0
MON%	1.1	%	0.0
GRA%	87.1	%	0.0-100.0
(EOS%)	1.4	%	00-100.0
LYM#	1.90	$10^3/mm^3$	1.00-4.00
MON#	0.10	$10^3/mm^3$	0.0-0.50
GRA#	14.70	$10^3/mm^3$	3.00-12.00
EOS#	0.23	$10^3/mm^3$	0.00-0.60
RBC	6.67	$10^b/mm^3$	5.00-10.00
HGB	10.0	g/dl	8.0-17.0
HCT	32.2	%	27-47
MCV	48	μm^3	40-55
MCH	15.0	pg	13.0-17.0
MCHC	31.1	g/dl	31.0-36.0
RDW	16.7	%	17.0-22.0
PLT	265	$10^3/mm^3$	180-430
MPV	11.4	μm^3	6.5-15

The patient was hospitalized for 24 hours prior to surgery. Buprenorfine 20 µg/kg and Meloxicam 0.2 mg/kg were given for pain management while maintained on fluids with Ringer solution at a rate 50 ml/kg/24 hours. Acepromazine 0.01 mg/kg + Buprenorphine 0.02 mg/kg was used for premedication. Induction was done with Propofol 3 mg/kg and maintenance with Isoflurane and oxygen. The patient was positioned in lateral decubitus, clipped and scrubbed with 4% chlorhexidine solution.

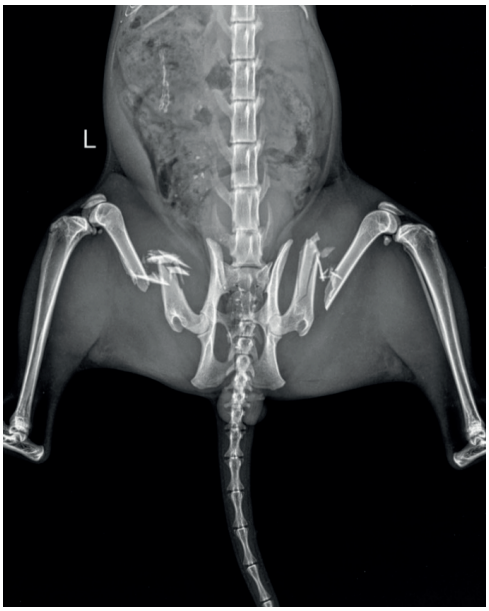

Figure 1. Initial radiological findings

Due to the severe comminuting aspect and the impossibility of obtaining an anatomic repair, it was decided to obtain stabilization using a system of "Tie-in" type external fixators.

The right hind limb was approached first through a lateral skin incision along the craniolateral region of the thigh. The Fascia Latta was then incised outlining the cranial edge of the *Biceps femoris*, the exposure of the diaphysis and the fracture site being achieved by caudal retraction of the *Biceps femoris* and cranial retraction of the *Vastus lateralis* muscle (Johnson et al., 2005).

After identifying the sciatic nerve, the proximal fragment was stabilized using two cerclage wires. While holding the hip extended and the femur adducted a Kirschner pin with a diameter of 2 mm and a length of 310 mm was inserted in a retrograde manner through the centro medullary canal perforating the trochanteric fossa, withdrawn to the edge of the fracture area and then inserted through the length of the entire femur after aligning the two fragments (DeCamp et al., 2016). A pin of the same length was used to assess the positioning of the implant in the distal fragment. The anatomical planes were repaired with PDS 2/0 absorbable monofilament suture material.

A total of 4 incisions of approx. 5 mm, 2 incisions proximal and 2 incisions distal to the fracture site for the lateral fixation of 4 Kirschner pins with a diameter of 1.6 mm. A 21G needle was previously used to puncture the soft tissues up to the level of the compact to avoid accidental injury to the neurovascular structures. A smaller 1.4 mm pin was used to engage at the most distal portion of the left hind limb for bone safety considerations. The pins were inserted using a rotational speed of 800 RPM at a sharp angle of roughly 70° (although it was not possible for all the pins in order to properly construct the apparatus) to the bone surface perforating both compacts while an assistant helped maintain a right angle between the greater trochanter and the patella (Piermattei et al., 2006). The free portion of the centro-medullary pin was bent at an angle of approx. 90° from the initial vertical plane and then bent once more at an angle of approx. 90° to the previously obtained horizontal plane and connected to the side pins using cerclage wire.
After assessing the bone alignment and mobility of the knee and pelvis, the external connection pin was covered with epoxy putty material ensuring a distance of approx. 2cm from the skin surface (Scott et al., 2022) The left hind limb was approached and stabilized in a similar fashion (Figure 2).

Figure 2. Radiological presentation after stabilization of the fragments with external fixators and epoxy putty

The checkup radiological examination confirmed a good alignment of the bone fragments. The patient began to bear weight immediately postoperatively and was discharged after 3 more days of hospitalization. While being under medical supervision it received Ceftriaxone 20 mg/kg/12 hours, Meloxicam 0.1 mg/kg/24 hours. It was only in the first 24 hours that contiuous rate infusion was given at a rate of 50 ml/kg/24 hours with Ringer solution and a suplement of Buprenorfine 10 µg/kg every 8 hours for better pain management. Treatment at home consisted of: Amoxicillin + Clavulanic acid (50 mg+12.5 mg)/12 h - 10 days; Robenacoxib 6 mg/24 h - 5 days; Iodine for the daily sanitization of components in contact with the skin. The patient returned for regular weekly check-ups.

Figure 3. Post-operative radiological appearance at 6 weeks

After 6 weeks, the radiological examination confirmed callus viability, which allowed for the removal of the implant (Figure 3). The pins were cut near the junction with the epoxy putty material and then mobilized by gentle left-right rotation movements.

RESULTS AND DISCUSSIONS

After the removal of the implant, both hind limbs could take over and support the patient's weight, moving effortlessly. No changes were observed in terms of joint range of motion.

Although the resulting callus obviously exceeds the normal bone silhouette with the inclusion of several fragments (Figure 3), the patient did not exhibit discomfort on palpation or manipulation.
The viability period of the implant depends on the loosening-up phenomenon, which involves the decrease of the contact surface between the bone tissue and the implant material, thus reducing its stability. This period can be extended by various methods such as the use of threaded pins, the complete perforation of both cortices and/or the application of hydroxyapatite on the contact surface of the implant with the bone (Fossum et al., 2018).
In the presented case, threadless Kirschner pins were used employing both cortices at a sharp angle to prolong the life of the implant and delay the loosening-up phenomenon (Piermattei et al., 2006)
Only ventro-dorsal exposures we're performed throughout the study as it was the best option to properly evaluate the healing process without the epoxy putty overlapping the femur.
On initial radiographic exposure the hind limbs we're not extended because of concerns about further injuring soft tissues and other neuro-vascular structures.
On the first postoperative radiological image (Figure 2), we could already observe some bending of the smaller size pin.
On the 6th week postoperative radiological image, we've decided to flex and abduct the right hind limb as to obtain a ventro-lateral exposure to better evaluate the cerclage repair used for the right femur (Figure 3). It's evident the left femoral external fixator, although retaining some bone contact, the smaller pin lost bone depth and migrated towards the lateral cortex losing stability because of its reduced stiffness.

CONCLUSIONS

It is generally recommended that a minimum of two pins must engage each fragment and an optimum of three to four pins for each may be used obtaining considerable stress reduction at the pin-bone site (Scott et al., 2022; Johnson et al., 2005). For this particular case only two pins were used for each fragment furthermore an intramedullary pin was added for better bending and compression support achieving an approximate normal bone length and alignment. This option also allowed for better pin stability as nonthreaded pins confer less retention force while at the same time cause less trans-cortical chip fractures compared to threaded pins (Anderson et al., 1993)
Epoxy putty once hardened makes it difficult for further adjustments and may require additional surgery if the device proves unstable after initial stabilization or during the patient's recovery.
Although this method of fracture reduction is not usually the first choice when it comes to excessively active patients, older or obese patients, and does require high owner compliance, it is up to each surgeon to properly assess each individual case as preoperative planning is key to obtaining the best possible outcome for each individual patient.
The main goal in orthopaedic surgery is to obtain early ambulation and optimum limb function. External fixators are a versatile reduction method offering surgeons numerous flexible options for the repair of long bone fractures. The device's frame can be modified and adapted so that it can effectively take over the mechanical forces of compression, tension, torsion, shear and bending involved for weight-bearing and natural movement.
We can thus recommend this as a viable and efficient method especially for the stabilization of feline femoral fractures with minimum to severe comminution, where an anatomical repair is not possible and a biological/functional repair is required instead.

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ASSESSING COMMUNICATION WITHIN COMPANION ANIMAL PRACTICES: VETERINARIAN VS. PET OWNER

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Abstract

Veterinarian-client communication has been the subject of many surveys, and guides were developed. This study assesses the communication patterns between veterinarians and pet owners in Romania. The study used an original questionnaire based on The Calgary-Cambridge Guide adapted for veterinary medicine. Following the analysis, it was concluded that, in most cases, the communication process was efficient. More than 90% of the owners felt encouraged to participate in the dialogue and appreciated that the veterinarian used an accessible language. The communication process included feedback, as 80.1% of the vets verified the owner's understanding. As negative aspects that influence the therapeutic relationship, we mention the fact that 21.6% of owners were not asked if they accepted the final plan, 38.6% were not informed about the costs during the consultation, and 57.3% did not receive a copy of the medical file at the end of the consultation. Communication, in most of the consultations, follows a biolifestyle-social pattern. However, the existence of the negative aspects that fall within a biomedical, authoritarian pattern supports the continuous need for communication education.

Key words*: communication skills, communication patterns, veterinarian-client communication.*

INTRODUCTION

Effective communication is a priority in human and veterinary health services, and its components are being studied and refined. Its components are health literacy, cultural skills, and language barriers (Ratna, 2019). Next, health literacy's impact on human and veterinary medicine will be presented.

Defined as the patient's ability to understand and draw conclusions based on reality and experience (Bash, 2007), health literacy can be successfully applied in medical services, disease prevention, and health promotion as a lifestyle (Sørensen et al., 2012).

The need for this form of competencies is supported by the large number of specialized studies, which are based on patients' observations: difficulties in understanding medical information, lack of knowledge that hinders communication, incorrect administration of treatments prescribed by the doctors, lack of preventive behaviours, and poor chronic disease management. These patients will be less involved in the dialogue with the medical team and decision-making process, with lower satisfaction (Koh et al., 2016).

The doctor is among the first facilitators of health literacy through doctor-patient interaction, improving the quality and manner in which he delivers the message to the patients and developing a correct and qualitative communication strategy among the medical team. Verbal information, doubled by a visual form (diagrams, charts), encouraging clarifying questions, and translating medical terms into accessible language are just some methods that can increase the patient's level of understanding with immediate and lasting effects on his health (Ratna, 2019).

Moreover, the use of empathy in the doctor-patient relationship brings benefits for both parties involved. The doctor will be more productive and efficient, have lower stress levels and depression rates, and have a better quality of life. The patient is more involved in the therapeutic relationship, is more treatment-compliant, and has improved chronic disease management (Moudatsou et al., 2020).

In veterinary medicine, the field of effective communication and health literacy is

developing. The methods and techniques developed for human medicine are transferable, the principles remaining the same. Higher education institutions have included in "Day one competences" the skill to communicate effectively with clients, the public, and the authorities (ECCT, 2015). It focuses on developing skills and abilities that the vets acquire during their graduation from the faculties of veterinary medicine and would further perfect them in practice.

The Royal College of Veterinary Surgeons reinforces the importance of communication and collaboration in professional relationships. The European Association of Establishments for Veterinary Education (EAEVE) has compiled a list of first-day competencies, describing effective communication as distinct (Lekeux & SOP WG, 2019).

Closed-ended questions and low levels of empathy prevent the owner from developing confidence with the veterinarian. The lack of encouragement to participate in the dialogue decreases the treatment compliance rate and directly affects the animal's health (Shaw et al., 2004).

The quality of communication influences the owner's attitude towards loyalty and satisfaction towards the veterinarian (Brown, 2018). Owners with a strong relationship with the vets tend to follow the recommendations more wholly and frequently than those who declare a weak relationship (Lue et al., 2008).

Veterinarians appreciate that communication skills are just as important as medical knowledge or skills (McDermott et al., 2015). The principles of effective communication applied within the medical team contribute to developing a harmonious, constructive working environment, and they increase the efficiency and effectiveness of the team (Pun, 2020). They also influence the rate at which owners turn to preventive medicine (Lue et al., 2008) or how they opt for prescribed procedures and treatments (Kanji et al., 2012).

There are three patterns of doctor-patient communication: biomedical, biolifestyle-social, and consumerist. The vet dominates the dialogue within the biomedical model, and the owner's contribution is reduced and discouraged. In the biolifestyle-social model, things are more balanced and oriented toward a therapeutic relationship in which both parties are involved and collaborate. The consumerist pattern shows the owner in a role of power, and the doctor is a simple consultant in the medical process (Shaw et al., 2006), (Shaw et al., 2008).

This study aims to qualitatively analyse the communication process between Romanian veterinarians and owners within companion animal practices. An analysis of the communication in this field is needed to assess the current level and propose solutions adapted to the population and the socio-cultural context.

Another significant milestone in veterinary-client communication was the COVID-19 pandemic, a period that demanded significant adaptability from the veterinary profession. Most clinics had to modify their protocols to align with national or state regulations, showcasing the resilience and resourcefulness of these professionals in the face of adversity.

During the COVID-19 pandemic, studies assessing the impact on veterinarian professionals have been conducted. In Romania, immediately after the lockdown, veterinarian professionals (vets, assistants, technicians, managers) reported how the lockdown affected their human resources, activity management, relationships between themselves and with the authorities, and how continuing education had changed. Also, they mentioned in the free-text comment box that they had difficulties communicating with the pet owners and that this relationship was affected (Mureşan et al., 2021).

The results may be valuable in every veterinarian clinic, as they can provide a better understanding of the relationship dynamics. Furthermore, they can be used in designing programs for students to improve their communication skills.

MATERIALS AND METHODS

a. Questionnaire Design

The qualitative research used a questionnaire addressed to pet owners. The questionnaire was created and processed using Google Forms and distributed through Facebook. Participation was voluntary, with respondents agreeing to the use and publication of data. The questionnaire link was distributed via online platforms, and

the exact Romanian region from which the respondents are is unknown.

The research team designed the original questionnaire, which the Bioethical Commission of the Faculty of Veterinary Medicine Bucharest approved. It contained 61 questions, divided into six sections. The first section collected general data about the owner, the species, the number of pets, and the frequency with which they visit the veterinarian. The other five sections are based on The Calgary-Cambridge Guide (CCG) (S. M. Kurtz, 2017) and collect data about: section no. 2 "Questions regarding the initiation of the consultation and information gathering", section no. 3 "Questions regarding the structure of the consultation", section no. 4 "Questions regarding the building of the doctor/owner relationship", section no. 5 "Questions regarding the amount and type of provided information" and section no. 6 "Questions regarding the type of provided explanations and planning".

Of the 61 questions, three are open-ended, 15 per 5-Point Likert Scale, 34 with a dichotomous answer, and eight with 3-5 answer options.

The CCG is part of a communication handbook dedicated to veterinary medicine (S. M. Kurtz & Adams, 2017), which aims to integrate communication skills acquired on the theoretical level with practical clinical skills in a single effective process (S. Kurtz et al., 2003). The vet/client communication process is divided into five stages to build the therapeutic relationship and structure the consultation.

b. Data Collection

Data collection was conducted from March 3rd to April 25th, 2021. All the data was stored using the Google Forms platform. Some restrictions were still in place during this period due to the COVID-19 pandemic, as the third wave reached Romania. The owners were asked to answer questions based on their vet's regular visits. From May 2020 through September 2020, many of the restrictions were revoked. In March and April of 2021, many businesses had a regular schedule with a few limitations, such as social distancing and the mandatory facemask.

c. Statistical Analysis

The IBM SPSS Statistics was used to analyse the collected data. Descriptive statistics were used to summarize the data and the following inferential tests: the Chi-Square Test of Independence, Pearson's Correlation Coefficient, and the Independent-Samples T-test.

For the data collected using the 5-Point Licker Scale, answers were converted into numeric values as follows: 5 = to a very large extent; 4 = to a large extent; 3 = to a moderate extent; 2 = to a small extent; 1 = to a very small extent.

RESULTS AND DISCUSSIONS

1. Demographic characteristics of respondents and pet ownership

The questionnaire had 171 respondents, all pet owners who participated voluntarily. Of these, 89.5% (n = 153) attended higher education, 9.9% (n = 17) had secondary education, and 0.6% (n = 1) had graduated from vocational school.

Respondents ranged between 22 to 64 years of age, distributed as follows: under 26 years of age - 9.9% (n = 17), 26-35 years of age - 53.2% (n = 91), 36-45 years of age - 27.5% (n = 47), 46-55 years of age - 8.2% (n = 14) and 56-64 years of age - 1.2% (n = 2).

Regarding gender, 84.2% (n = 144) identified themselves as female, 15.2% (n = 26) as male, and 0.6% (n = 1) as non-binary.

Distribution and frequency of pet ownership: 45% (n = 77) - one animal, 21.2% (n = 36) - two animals, 12.3% (n = 21) - three animals, 9.9% (n = 17) - four animals, 3.5% (n = 6) - five animals and 8.2% (n = 14) - more than five animals.

A percentage of 39.2% (n = 67) had cats, 31% (n = 53) had dogs, and 24.6% (n = 42) had both species; the remaining 5.2% (n = 9) had other species. 84.2% (n = 144) had a regular veterinarian. The frequency of vet visits was as follows: 32.2% (n = 55) - twice a year, 30.4% (n = 52) annually, 24.6% (n = 42) every three months, and 12.9% (n = 22) - monthly.

Given that most pet owners, 84.2% (n = 144), have a current veterinarian that they visit frequently, it is safe to assume that the answers provided to the following questions are based on experience.

2. Results regarding the consultation structure, information gathering, building up the doctor/owner relationship, and providing information and planning.

The waiting time in the office until the takeover was relatively short, a maximum of 10 minutes for 63.7% (n = 109) of the respondents. For 29.8% (n = 51) of the cases, the waiting time was about 15 minutes, 5.3% (n = 9) about 30 minutes, and 1.2% (n = 2) more than 30 minutes. This aspect is relevant for professionalism assessment, given that most consultations require an appointment.

The first step in building a good quality therapeutic relationship is the initial meeting, first contact. In this direction, 40.4% (n = 69) of the owners said that the vets did not introduce themselves (name and position), 42.1% (n = 72) answered affirmative, and 17.5% (n = 30) did not remember this aspect. Related to this first step, 66.1% (n = 113) said that the doctor knew their name and their pet's name, 28.7% (n = 49) answered negative, and 5.3% (n = 9) did not remember. There is a significant association between the two variables; vets who introduce themselves are likelier to know the names, $\chi 2 = 37.40$, df = 1, N = 136, p <.001, with a Cramer effect size coefficient, $\phi = 0.52$. The subjects who answered "I do not remember" were excluded. This attitude increases respect and confidence from the owner's perspective.

One of the components of an effective communication process is encouraging dialogue. At the beginning of the consultation, 95.9% (n = 164) of the owners were asked to list all the aspects they wanted to discuss, and 94.7% (n = 162) were listened to carefully, without interruptions, behaviours within the biolifestyle-social model.

In 13.5% (n = 23) of the cases, the doctor did not ask for clarifications or the amplification of certain answers that were unclear. In 69.6% (n = 119) of the cases, he summarized the aspects presented by the owner, and in only 58.5%, the vet did a summary after every problem/topic of discussion. Summarization is an effective method proposed by the CCG that the doctor can use to ensure that he has understood correctly the information received or to emphasize the consultation structure for the owner.

In 98.8% (n = 169) of the situations, the doctor used accessible language, comments, and simple questions, and in 90.6% (n = 155), he explained certain medical terms in everyday language. Most clients, 96.5% (n = 165), consider the consultation timing effective. Regarding the consultation structure, only in 11.7% (n = 20) of cases did the owners not realize when the doctor addressed another topic or problem of the animal.

A good therapeutic relationship, as mentioned earlier, contributes to the owner's satisfaction (Brown, 2018) and increases the likelihood that he will follow the instructions he receives (Lue et al., 2008). 70.8% (n = 121) of the owners consider that the doctor paid attention to their emotional state and showed self-confidence in 99.4% of the cases. 91.2% (n = 156) felt that their ideas were accepted without feeling judged. Sensitive and shameful subjects were approached with empathy and understanding in 93.6% of cases (n = 160). These results placed the relationship in a biolifestyle-social model.

From the owners, 42.7% (n = 73) felt that their ideas were explored to a very large extent, 29.8% (n = 51) to a large extent, 21.6% (n = 37) to a moderate extent, 4.7% (n = 8) to a small extent, and 1.2% (n = 2) to a very small extent. Regarding their limits (time, treatment schedule, finances), 47.4% (n = 81) consider that were taken into account to a very large extent, 24% (n = 41) to a large extent, 15.8% (n=27) to a moderate extent, 9.4% (n = 16) to a small extent, and 3.5% (n = 6) to a very small extent. There is a strong positive correlation between these two variables, r (169) = .67, p < .001, indicating that the vets who explore the client's ideas also consider their limits (Figure 1).

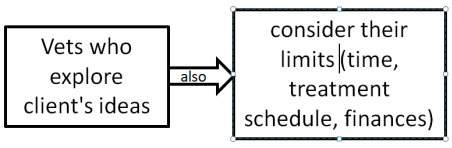

Figure 1. Strong positive correlated behaviours

There are strong positive correlations between three behaviours the vet can manifest regarding the communication process (Figure 2). The vets who share their decisional process also share the reasoning behind some questions or

manoeuvres, r (169) = .70, p < .001; they also explain the procedures and results of the physical exam, r (169) = .70, p < .001. Also, there is a strong positive correlation between the vets who expose the reasoning behind some questions and those who explain the procedures or the results, r (169) = .71, p < .001.

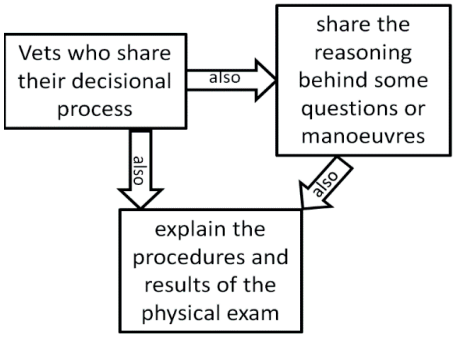

Figure 2. Three vet behaviours that correlate

More than half of the vets, 64.9% (n = 111), consulted with the owner about the case management options. In 42.7% (n = 73), the vet makes sure that the owner has the willingness and the available resources to follow the treatment to a very large extent, in 18.1% (n = 31) to a large extent, in 19.9% (n = 34) to a moderate extent, in 7.6% (n = 13) to a small extent and in 11.7% (n = 20) to a minimal extent. The vets who consulted with the owner about case management (M = 4.25, SD = 1.07) also made sure about the willingness and the available resources compared with those who do not display these behaviours (M = 2.75, SD = 1.38), t (98.7) = 7.35, p < .001.

From the vets, 57.9% (n = 99) offered information about treatment options (names, steps, advantages and disadvantages) to a considerable extent, 24.6% (n = 42) to a large extent, 10.5% (n = 18) to a moderate extent, 3.5% (n = 6) to a small extent, and 3.5 % (n = 6). This aspect positively correlates with the assurance about the willingness and available resources, r (169) = .66, p < .001.

These interrelated attitudes confirm that once the vet learns and admits that the owner has to be a partner in the process, new ways of inclusion appear, and constant negotiation must happen (Figure 3).

"Chunks and checks" is a technique described in the CCG (S. M. Kurtz & Adams, 2017) as giving information in small chunks and checking for understanding. Of most vets, 92.4% (n = 158) gave information in manageable chunks, 80.1% (n = 137) checked for the owner's awareness, and 68.4% (n = 117) asked if the owner needed other information regarding the causes, prognostics, or evolution. Checking for understanding is a form of asking for feedback.

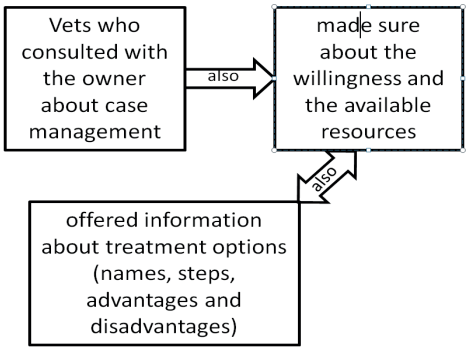

Figure 3. Vet interrelated attitudes

Checking for understanding is a form of asking for feedback.

Regarding the vet's ability to signpost and provide structured information, 74.9% (n = 128) of the owners estimated that the essential aspects were signalled and divided into categories. 87.1% (n = 149) considered that the explanations were organized. The consultation was usually structured, and the explanations were organized. For the owner, these aspects facilitate a better understanding of the process, which will positively influence his level of health literacy and care from which the pet can benefit.

Speech supported by visual methods increases the chances of accurate recall and better understanding. Following the analysis of the responses, in 76.6% (n = 131) of cases, no visual methods were used during the explanations. Despite that, 58.9% (n = 100) of the owners considered that by the end of the consultation, all the essential aspects related to the diagnosis and the treatment plan were clarified to a very large extent, and 30.4% (n = 52) to a large extent.

Several aspects negatively impacting the communication process and therapeutic relationship were identified. It has been found

that 21.6% of the owners (n = 37) were not asked if they accepted the final plan, 38.6% (n = 66) were not informed about the costs during the consultation, and 57.3% (n = 98) did not receive a copy of the medical file at the end of the consultation, neither in print nor in electronic format (Figure 4).

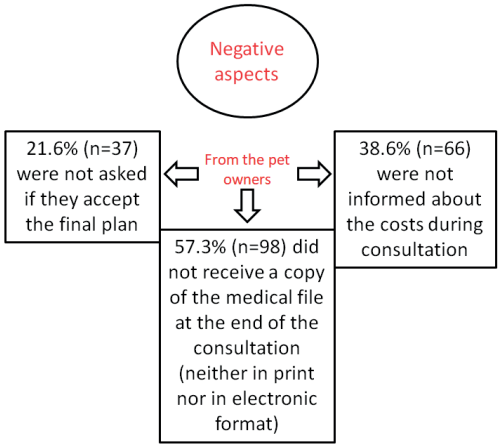

Figure 4. Aspects with a negative impact on communication and relationships

The vets who inform the owner about cost also tend to make sure about his willingness and available resources (M = 4.26, SD = 1.03), compared to those who do not inform (M = 2.88, SD = 1.46), t (105.35) = 6.69, p < .001. Moreover, the vets who inform about costs offer more information about treatment options (M = 4.67, SD = .615) as opposed to those who do not inform about cost (M=3.71, SD=1.26), t (84.67) = 5.73, p < .001. The interactions mentioned above support the partnership of the biolifestyle-social model (Figure 5).

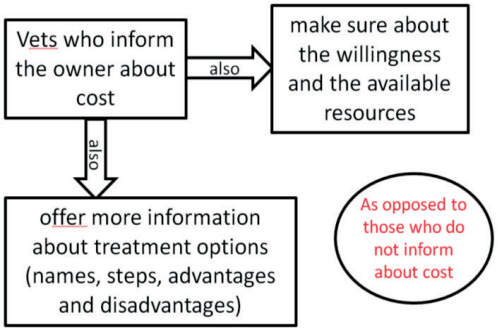

Figure 5. Interactions of the biolifestyle-social model

The negative aspects identified in this study influence the therapeutic relationship. The fact that the owner was not asked if he accepts the final plan frames the relationship in a biomedical communication model, in which the doctor has an authoritarian parental attitude and does not aim to create an alliance with the owner (Shaw et al., 2006; Shaw et al., 2008).

The financial component must be included in planning case management. The owner must evaluate his resources and agree to invest them, as well as reasons for further information and consent. Even though financial resources have a significant role in treatment management, they have less influence on the poor follow-up of recommendations than ambiguity, misunderstanding, or confusion (Lue et al., 2008). Thus, it is recommended to consider the owner as a partner who makes the final decision.

Written information, which the owners can use anytime for unclear issues or to make sure that they follow the complete steps of the recommendations or treatment, leads to better results of the medical process for the better care of pets. A study by the American Animal Hospital Association confirms that owners who had received written information at home used it in a reasonably high percentage (Association, 2009). The ability to verify the information influences treatment compliance (Englar, 2020). Owners who receive clear recommendations and explanations are seven times more adherent to following prescribed procedures (Kanji et al., 2012).

In the last section, "Comments, suggestions or anything else you think is relevant and has not been included" none of the owners mentioned COVID-19 pandemic limitations or influence in their vet visits. Most of the answers regarding time usage, communication patterns, and type of vet-pet owner relationship were positive, indicating little or no influence of the few limitations still in place during March and April of 2021.

CONCLUSIONS

Following the analysis of the answers received, it has been concluded that in most cases, the communication process was efficient; more than 90% of the owners felt encouraged to

participate in the dialogue and appreciated that the veterinarian used an accessible language. In 92.4% (n = 158) of the cases, the owners appreciated that information was provided in small quantities. The communication process included feedback, as 80.1% (n = 137) of the vets verified the owner's understanding.

The owners who participated in the study appreciate their relationship with the veterinarian as being good at communication and providing medical information. Over 80% of owners positively appreciated the veterinarian and his various communication skills, and 88.9% (n = 152) considered that all the essential aspects of the diagnosis and treatment plan had been clarified by the end of the consultation.

The percentage of owners who received a medical record at the end of the consultation was small, reaching 42.7% (n = 73). The lack of this practice negatively influences treatment compliance.

Communication, in most of the consultations, follows a biolifestyle-social pattern. However, the existence of the negative aspects that fall within a biomedical, authoritarian pattern supports the continuous need for communication education. It is safe to assume that once an attitude or behaviour is formed and maintained, and the benefits are more noticeable, other behaviours from the same cluster may appear with the right motivation. Education and communication training are needed for vets with experience and students or new graduates.

The topic of communication between the veterinarian and the pet owner is more and more common among professionals in Romania. This study is considered the first in this country on communication issues in companion animals' veterinary practices.

As strong points, this study can be a template for future research within this field and creates an overview that provides direction for more detailed approaches.

LIMITATIONS OF THE STUDY

A unilateral point of view is presented regarding the aspects to be improved, meaning that only pet owners provided their opinions. In this case, the research could be extended among veterinarians for a complete communication process analysis. It is also necessary to differentiate between urban and rural environments.

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ADVANCEMENTS IN LAPAROSCOPIC SURGERY FOR VETERINARY MEDICINE: ESSENTIAL INSTRUMENTS AND PROCEDURES

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Abstract

In recent years, laparoscopic surgery has gained from numerous technological advancements, that have improved results and decreased surgical complications. The availability of a wide variety of equipment and instruments enables the performance of surgical procedures, avoiding the need for extensive incisions that are common in the field. Laparoscopic surgery is an expanding domain of knowledge within the veterinary profession, with patients being the primary beneficiaries. Laparoscopic and laparoscopic-assisted procedures are well established in veterinary surgery, with novel minimally invasive approaches and procedures described regularly in the peer-reviewed literature. It is expected that as this field progresses, the benefits associated with accessibility, visualisation, and magnification will continue to demonstrate the superiority of laparoscopic and laparoscopic-assisted procedures over conventional open surgery for specific procedures. This article describes the essential instruments required to perform laparoscopic surgical procedures and examines several laparoscopic procedures that have been implemented in the field of veterinary medicine.

Key words*: laparoscopy, surgery, minimally-invasive-surgery, veterinary medicine.*

INTRODUCTION

Minimally invasive surgery has gained significant popularity in veterinary medicine due to its various benefits, including reduced hospitalization time, faster recovery, decreased stress and discomfort, and improved visualization of abdominal organs (Peteoaca A. et al., 2017).

Hippocrates (460-375 B.C.) conducted the initial endoscopic studies and documented a retroscopy tool that resembles modern-day ones. In 1905, G. Kelling conducted the first laparoscopic surgery on a canine subject, developing an insufflation technique to enhance visualization. In 1913, Jacobeus introduced and carried out the minimally invasive procedure in human medicine, separate from Kelling (Mayhew P., 2011). The inaugural laparoscopic procedure in veterinary medicine took place in 1985, involving the sterilization of a female dog through the closure of the uterine horns (Case JB et al., 2011).

Laparoscopic surgical procedures are recognized for their minimal invasiveness, low incidence of complications, and low death rate. The utilization of contemporary laparoscopic procedures enables the reduction of patients' hospitalization duration, ensuring accelerated recovery and resulting in increased satisfaction among patients (Doerner J. et al., 2012).

Laparoscopic surgery, being a minimally invasive treatment, is thought to decrease the neuroendocrine, immunologic, and metabolic response of the body in comparison to laparotomy surgery, owing to modest tissue injury (Freeman, 1999).

Furthermore, there has been a rise in the quantity of emergency surgeries conducted using laparoscopic techniques. Laparoscopy is used for a variety of purposes, including biopsies of nearly all organs that can be accessed through laparotomy (liver, spleen, pancreas, lymph nodes, kidney, adrenal gland, cholecystocentesis, prostate) (Monnet E et al., 2003). In addition, laparoscopy is a minimally invasive approach for conducting a range of surgical procedures, with the number of procedures performed increasing as experience and expertise grow (abdominal exploratory, ovariectomy, ovariohysterectomy, and/or ovarian remnant removal, abdominal cryptorchid testicle removal, adrenalectomy,

cholecystectomy, liver lobectomy, splenictomy, nephrectomy, artificial urethral sphincter placement, cystotomy and urethrocystoscopy, etc) (Fransson B.A. et al., 2010).
Supplementary surgical procedures (gastrostomy tube, cholecystostomy tube, jejunostomy tube, cystostomy tube), such as the placement of feeding tubes to aid in recovery or to stabilize patients prior to procedures, can also be carried out, as well as a comprehensive examination of the abdominal region for oncologic staging purposes (Micsa C. et al., 2019). The use of laparoscopy offers several advantages over traditional laparotomy, including improved visualization of organs and pathology due to magnification and illumination. This allows for more precise biopsies of specific lesions, resulting in the collection of larger samples than could otherwise be obtained through percutaneous methods (Freeman L.J., 2009). Compared to laparotomy, laparoscopy is associated with decreased patient morbidity, pain, infection rates, and recovery time (Buote N.J. et al., 2011). Additionally, laparoscopy offers the ability to document the pathology of organs, which is beneficial for developing treatment plans, medical record-keeping, monitoring chronic conditions, and educating clients and veterinary colleagues involved in the care of patients (Micsa C. et al., 2018).

MATERIALS AND METHODS

The preoperative patient preparation for minimally invasive surgery of the abdomen shares numerous parallels with standard open abdominal surgery. Preparation involves following a regular preoperative fasting practice and administering perioperative antibiotic prophylaxis based on individual circumstances.
Additional preparatory measures include emptying the urine bladder and making a broader incision in the hair than what would be done for a typical ventral midline laparotomy. Emptying the urine bladder creates more room in the peritoneal cavity during the laparoscopic surgery and reduces the chance of unintentional bladder injury when creating laparoscopic portals. A broader hair clip enables the insertion of laparoscopic portals in a more sideways position, which helps to achieve the desired instrument triangulation (Draghici I et al., 2015).

Laparoscopy principles
In the performance of minimally invasive procedures, several key principles and tactics are essential. Before commencing any surgical intervention, it is necessary to carefully plan and consider the patient's placement, the positioning of the tower, the arrangement of the surgical team, and the location of the instrument table.
The positioning of the patient during minimally invasive abdominal surgery is primarily determined by the specific treatment being performed. A surgeon can utilize the force of gravity to passively retract the abdominal viscera by changing the patient's position. This helps in providing better visibility of the specific anatomical components required for a particular surgical treatment. Throughout the treatment, it may be necessary to alter the posture, therefore necessitating an adjustable operating table capable of accommodating the needed angles. When performing procedures in the retroperitoneal region, it is beneficial to place patients in a lying posture with their chest down and their pelvis supported. This allows the abdominal organs to naturally move away from the retroperitoneal structures that are being examined.
An optimal operating table designed for minimally invasive surgery enables lateral tilting of the table to facilitate sequential access to each side of the reproductive tract. Additionally, it allows tilting of the front and rear ends of the table, such as the Trendelenburg position, to maximize exposure to the caudal abdomen. Securing the meticulous attaching of patients to the table is crucial in order to avoid any unintended slipping or falling incidents while carrying out the surgery. Table top add-on patient positioning systems are increasingly prevalent in the veterinary laparoscopic supplies industry. These devices provide the capability to modify a pre-existing surgical table that does not tilt, in order to make it suitable for laparoscopic procedures.
The positioning of the tower is of utmost importance for ensuring efficient and seamless

surgical procedures. It is crucial that the tower and the monitor be aligned directly with the surgeon's body and the angle of the telescope.

The surgical technique used for minimally invasive abdominal surgery differs based on the specific treatment being performed. The number of intended portals for a certain surgery can vary depending on the surgeon's discretion, which may determine modifications to the precise positioning of the port. Typically, numerous laparoscopic operations involve the use of portals positioned on the front side of the abdomen in a layout resembling a baseball field. This arrangement aids in the precise positioning of equipment in a triangular formation. It is vital to examine alternative methods to improve the visibility of specific organs during certain procedures (Usón J. et al., 2010).

For instance, a paralumbar technique can be employed for adrenalectomy and significantly enhances the visibility of the gland during the operation (Jimenez P. et al., 2008). The use of a 0" telescope that is put into a screw-in threaded trocar during its establishment is optimal for directly identifying the tissues or organs towards which the port is progressing.

Instruments are usually identified by their extended shape and length and varied use. The instruments are introduced into the body through specific hermetically sealed openings with a diameter of either 5 or 10 mm. These ports function to protect the devices and guarantee uninterrupted access. The equipment includes specialised tools for performing intracorporeal suturing, grasping, cutting, and tissue sampling (Peteoaca et al., 2018). To effectively manage bleeding, various specialist sutures and hemoclips can be utilised with little invasiveness. Alternatively, there has been an increased frequency in the creation of several distinct vessel sealing devices. Two examples of these devices include the Ligasure (Covidien), which use electrocautery, and the Harmonic scalpel (Ethicon), which employs ultrasonic technology.

Developing expertise in positioning the surgical team and the operating instrument table is a talent that is honed through practical practice. The ideal team positioning will differ for each process (Tanase A et al., 2015).

Although laparoscopy is often seen as a less invasive procedure, it may not be appropriate for every patient. Laparoscopy should not be performed in patients who have diaphragmatic hernia and significant intra-abdominal adhesions. Avoid doing laparoscopic procedures in animals that are obesse, have respiratory problems, or are in a generally unhealthy condition. Elderly animals may also have an increased probability of encountering challenges when undergoing general anaesthesia in conjunction with pneumoperitoneum. Patients having a history of prior abdominal surgery have an increased difficulty when performing laparoscopy. While pyometra is typically seen as a reason to avoid laparoscopy, there have been documented cases of laparoscopy-assisted ovariohysterectomy being performed successfully in two female dogs with pyometra.

RESULTS AND DISCUSSIONS

Laparoscopic procedures

Before doing laparoscopy on a patient, it is essential to ensure that they are restricted from eating for 12 to 24 hours. The animal must be provided with fresh water throughout the fasting period, except for the 4-hour interval directly preceding the treatment. The urinary bladder, colon, and stomach should be empty of any substances or materials (Lansdowne JL et al., 2012).

Prior to any medical intervention, the abdominal cavity is filled with carbon dioxide gas (CO_2) in order to create a pneumoperitoneum (Catalin M. et al., 2015). This provides sufficient room for the instruments and camera, as well as improved visualisation. Carbon dioxide is advantageous because of its non-combustible properties, affordability, lack of colour and aroma, and high solubility. Consequently, it is rapidly assimilated throughout the entire body following the procedure. Helium gas can be used because of its inert properties, despite its high cost.

Common sites for positioning portals in surgery include the umbilicus for the camera, the ventral midline for instrument ports, and the paramedian area for instrument ports (Sánchez-Margallo F.M. et al., 2007). Portals often have

a diameter of either 5 or 10 mm, and the majority of procedures necessitate the use of 3 to 4 ports.

In the past few years, there have been significant progressions in the implementation of single-point access methods. The procedures are carried out using a specific port called a single incision laparoscopic surgery (SILS) port, which is made by Covidien. These processes employ specialised equipment with flexible components and joints to improve the precision of triangulation (McClaran J.K. et al., 2009).

The following protocols are commonly performed on small domesticated animals:

Laparoscopic ovariectomy, ovariohysterectomy, or ovarian remnant removal

Laparoscopic ovariectomy or ovariohysterectomy is a frequently performed surgery that serves as an initial step for many veterinary surgeons in their laparoscopic practice. The benefits of using a modern technique instead of a classic open approach include improved visualization and quicker recuperation time (Austin B. et al.; 2003).

Patients are positioned in a dorsal recumbency position on a table that can be inclined to the left or right. Various procedures, including 1-, 2-, and 3-port approaches, have been documented. The 1-port approach utilizes a 10-mm operating scope with a 5-mm instrument channel to perform procedures. A widely used method involves using a 2-port technique, in which a port is positioned below the umbilical area, and a second port is positioned either above or below the first port.

Subsequently, it is attached to the body wall using a percutaneous swaged-on needle with a suture or a laparoscopic hook. An ovarian removal procedure can be performed using a vascular sealant device. If the ovary is secured with a suture, it can be left in position and removed once the opposite ovary is removed, or it can be removed right away if a laparoscopic hook is utilized. A 3-port approach can be implemented by positioning all 3 ports along the midline, with 2 ports located below the umbilicus and 1 port positioned above the umbilicus. This technique does not require the suspension of the ovary from the abdominal wall.

The 3-port approach is a viable method for conducting an ovariohysterectomy. The ovarian pedicles on both sides are cut and the wide ligament is also cut around the uterus to reduce the amount of blood vessels and lessen the risk of harm to the ureters and gastrointestinal tract using electrosurgical equipment. This procedure is conducted in a cranial to caudal direction, while maintaining continuous tension on the appropriate ligament (Dupre G. et al., 2009).

The ovaries and uterus are brought outside the body through the incision at the tail end, where the main part of the uterus is tied off and cut in a standard way. problems encompass bleeding and other typical laparoscopic problems. The 3-port procedure can also be used to do an ovariohysterectomy for pyometra (Adamovich-Rippe et al., 2013). A wound retractor device can be utilized at the caudal portal to assist with the extraction of the uterus. It is advisable to carefully choose the cases, following rules for dogs weighing less than 10 kg and having a uterine horn diameter smaller than 2 cm, or dogs weighing more than 10 kg and having a uterine horn diameter smaller than 4 cm. Possible consequences include uterine rupture and bleeding.

A recent study demonstrated that dogs who received laparoscopic ovariohysterectomy required a lower dosage of analgesic medication post-surgery in comparison to those who had an open laparotomy. In 2009, Culp W.T. et al. conducted a study which revealed that laparoscopic surgery in small dogs led to a lower decline in postoperative activity levels as compared to open surgery.

Cryptorchid neutering

Laparoscopic assessment of the peritoneal cavity can be beneficial in both diagnosing and treating abdominal cryptorchidism through either a complete laparoscopic or laparoscopically-assisted approach (Mayhew P., 2009). Laparoscopic surgery has several advantages over conventional surgery, including facilitating the search for the abdominal testicle, reducing the duration of the procedure, and promoting faster recovery in the animal. Employing a retrieval bag for the removal of the abdominal testicle is particularly

advisable when there is any concern for testicular neoplasia.

Gastropexy is a preventive technique that aims to avoid the development of gastric dilatation volvulus (GDV), or to prevent its return upon surgical repair (Dorfelt R. et al., 2012).
Laparoscopically-assisted gastropexy is a highly effective procedure that combines the benefits of a minimally invasive method for safety and the traditional open suturing technique to reduce the time required for the operation. Furthermore, the combination of prophylactic gastropexy and routine ovariectomy, both conducted exclusively through laparoscopy, has demonstrated a significant success rate and little occurrence of complications for dogs who are prone to GDV (Rivier P. et al., 2011). Recently, a technique called laparoscopic gastropexy has been developed. This technique involves using a single port for access and using specialized equipment and telescopes that can move and bend at different angles. Some surgeons prefer laparoscopic-assisted gastropexy due to its technical simplicity and the fact that treatment does not necessitate specific equipment beyond a standard laparoscopic setup.

Laparoscopic-assisted Cystoscopy
The growing prevalence of laparoscopy in diagnostic and therapeutic urologic procedures is remarkable. Nevertheless, it is crucial to highlight that laparoscopically-assisted cystotomy should not be carried out when there is suspicion of transitional cell carcinoma (Defarges A et al., 2013). This is because there is a significant probability of aggressive abdominal metastases occurring if the bladder is breached.
Vesicular calculus is the primary reason for doing cystotomy in dogs. In addition, laparoscopic technique may be beneficial for cases of chronic cystitis that do not respond to medicinal therapy, as well as for the removal of mineral plaques or ulcerated areas (Rawlings C.A. et al., 2003). Moreover, urethrocystoscopy can be utilized to examine a broad spectrum of disorders impacting the lower urinary and reproductive tracts (Zhang J.T., 2010).

Procedure for obtaining liver, renal, spleen and pancreatic tissue samples using laparoscopic techniques
If it is feasible to do abdominal exploratory and organ biopsy using minimally invasive surgery, this approach is preferred over alternative procedures.
There are numerous options for collecting tissue samples, and both laparoscopic and laparoscopically-assisted methods provide a less invasive option for biopsying various organs in the lower abdomen and the thorax (Petre S.L. et al., 2012).
Liver and spleen biopsies are frequently performed for diagnostic purposes using laparoscopy in small animal practice, particularly in situations with hepatic lesions, diffuse diseases, and splenomegaly.
Advantages over blind approaches include the capacity to visually perceive the surface texture and color, the capability to select a precise puncture site, and enhanced control over any potential hemorrhage. Furthermore, it is possible to obtain bigger diagnostic biopsy samples compared to those collected using ultrasound guided spring loaded biopsy needles.
The indications for pancreatic biopsy are to distinguish between acute pancreatitis and acute liver illness, as well as to visualize both organs. In addition to serving as a means of collecting tissue samples, laparoscopic procedures offer the benefit of enabling the surgeon to examine the pancreas in relation to other surrounding organs. By conducting laparoscopic explorations, the most suitable site for biopsy can be identified, thereby minimizing the likelihood of damaging the pancreatic duct (Radhakrishnan A. et al., 2013).
Renal biopsy serves as a valuable diagnostic tool in primary renal disease and in assessing the extent and severity of renal involvement in other systemic disorders. Compared to the traditional blind technique, laparoscopy offers several advantages, including direct visualization of the kidney post-biopsy and the ability to evaluate and control for hemorrhaging (Nowicki M. et al., 2010).
Laparoscopy enables the examination of internal organs and the visual confirmation of hemostasis, without the requirement for invasive open surgery (Micsa C. et al., 2017).

Cholecystectomy.
Laparoscopic cholecystectomy is commonly used for cases of simple gallbladder mucoceles. Nevertheless, it is advisable to refrain from using it in instances of intricate mucoceles, such as coagulopathies, bile peritonitis, extra-hepatic biliary tract obstruction, or when dealing with patients of tiny body size (weighing less than 4 kg) (Micsa C. et al., 2015).
For the surgery, the patient should be positioned lying on their back, and a technique using four ports should be used. These ports include one below the navel, one in the upper left part of the abdomen, and two in the upper right part of the abdomen. These ports are arranged in a triangular pattern around where the gallbladder is expected to be located. The surgeon should assume a Trendelenburg posture, while using a fan retractor in the left port (Lansdowne J.L. et al., 2012). The laparoscope should be placed in the right-sided port closest to the midline, while the other right port, along with the subumbilical port, should be used for instruments controlled by the surgeon (Pascal M. et al., 2015).
It is important to meticulously dissect the cystic duct just before the first hepatic duct and then secure it by either using hemoclips or suturing. Next, the gallbladder should be carefully separated from the hepatic fossa. If there is any occurrence of bile leakage or hemorrhage, it is necessary to take an open approach. It is necessary to enclose the gallbladder in a specimen retrieval bag before removing it.
It is crucial to have knowledge of potential complications linked to the procedure, such as cystic duct rupture, confusion between the cystic and common bile duct, and bile spilling resulting from the cystic duct ligation. In order to reduce these hazards, it is advisable to secure the cystic duct by tying it twice using a single-strand suture, either with knots made outside or inside the body. Moreover, it is advisable to conduct a liver biopsy to get bacterial culture and histology samples, in addition to a bile culture.

Laparoscopic-assisted enterotomy.
Typically, in medical practice, a laparoscopically-assisted technique involves using laparoscopy to grasp on the colon and bringing a section of the bowel outside the body before making an incision to remove the foreign object. This procedure involves making an incision in the intestinal wall to examine the mucosa and remove any foreign objects that are blocking the intestinal passage. It can also be used to take biopsies that penetrate through the entire thickness of the intestinal wall (Gower S.B. et al., 2011). Afterward, the incision in the intestine is closed with sutures. Due to the nature of the procedure, which involves exposing the contents of the bowels, the surgeon must exercise caution to avoid peritonitis. Additionally, the surgeon should possess a comprehensive set of advanced abilities (Catalin M. et al., 2015).

Additional laparoscopic or thoracoscopic procedures
Laparoscopy and thoracoscopy offer a wide range of applications, both for diagnostic and therapeutic treatments. Some more treatments that can be performed via laparoscopy include diaphragmatic hernia repair, cholecystectomy, nephrectomy, and transperitoneal or retroperitoneal lymphadenectomy for cancer staging (Lansdowne J.L. et al., 2005).
Thoracoscopy enables the performance of several treatments, including closure of persistent ductus arteriosus, drainage of chylothorax, and ligation of the thoracic duct (Kim Y.K. et al., 2013).

Thoracoscopy procedures
This is among the most beneficial minimally invasive surgeries (MIS) strategies utilized in small veterinary practices, as it enables the surgeon to execute procedures that are comparable to those carried out via open thoracotomy (Sakals S. et al., 2011).
In addition, thoracoscopy is a minimally invasive surgical procedure that applies a thoracoscope to enable direct visualization and examination of the thoracic cavity and pleural space. Tumour resection can also be accomplished via minuscule incisions, as opposed to open chest surgery, which is renowned for its substantial morbidity and protracted recuperation period. It is important to note that thoracoscopy should only be performed by surgeons who possess prior knowledge and expertise in open thoracic surgery techniques.

CONCLUSIONS

Laparoscopy is a technique that enables the execution of various diagnostic and surgical procedures with minimal invasiveness. Laparoscopy, when executed by a proficient surgeon, is a direct, safe, and efficient technique for performing surgical procedures in animals.

Thoracoscopy and laparoscopy feature a wide range of therapeutic and diagnostic applications. We highlight the following additional procedures that can be performed via laparoscopy: cholecystectomy, nephrectomy, diaphragmatic hernia correction, transperitoneal or retroperitoneal lymphadenectomy for cancer staging, and nephrectomy. Additionally, thoracic duct ligation, drainage of chylothorax, and persistent ductus arteriosus ligation are all procedures that can be performed via thoracoscopy.

Occasionally performed surgeries include oncologic staging/biopsies, splenectomy, intestinal resection and anastomosis, intestinal foreign body removal, portosystemic shunt ligation, and feeding tube installation.

Complications can occur during laparoscopy. Haemorrhage is commonly reported, with a prevalence ranging from 20% to 43% (Micsa C. et al., 2015). Typically, vascular sealant devices are used to manage them, and only in rare cases is it necessary to switch to an open surgical approach. Organ damage, specifically to the spleen, can occur when entering the abdomen prior to insufflation, with a frequency ranging from 3-18% (Mayhew P 2011). However, it rarely requires specific medical intervention. The conversion rate from laparoscopy to laparotomy is approximately 10-20%. Factors that increase the probability of transitioning to an open surgical method include being diagnosed with cancer, having a greater body weight and body condition score, and the surgeon's level of expertise.

It is important to mention that many of these essential challenges associated with MIS are strongly linked to the lack of expertise of the surgeon and their team, with a higher occurrence during the initial stages of the learning process (Fransson B.A. et al., 2012).

Minimally invasive surgical techniques are gaining popularity because they offer several advantages over open surgery for a wide range of procedures. The continual stimulation of further inquiry into the uses of this treatment for specific disease conditions will continue due to the drop in illness rates and the increasing demand from clients. Laparoscopic techniques provide a viable alternative to traditional approaches. The growing popularity of laparoscopic operations in veterinary medicine is due to their low invasiveness, quick hospitalisation, and fast recovery time for animals. This has made them attractive to both veterinarians and pet owners.

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DEVELOPMENT OF A MURINE MODEL OF NEUROBORRELIOSIS INDUCED BY HUMAN *BORRELIA* STRAIN

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Abstract

The genus Borrelia is represented by spirochetes that, once entered the human or animal body, can migrate to different organs, including the central nervous system. The aim of the study was to develop a murine neuroborreliosis model by inoculating a strain of Borrelia burgdorferi (Bb). 2 studies were conducted using BALB/c and CD1 mice strains, female, 8 weeks old, 22 grams at baseline. The selected animals were inoculated with Bb (10^6 spirochetes/mL), depending on the protocol, intradermally (100µL/animal) or intracerebrally (10µL/animal). For the evaluation of the neuroborreliosis development, endpoints were established at 1, 3, 6, 8 and 13 weeks post-infection, when samples of dura mater, brain, ear, heart, knee joint and bladder were collected, and maintained in culture medium specific for the growth of Borrelia. Recovery of Bb was possible from samples harvested 7 days post-infection from animals inoculated both intradermally and intracerebrally. Through the experiments we have developed a model of neuroborreliosis in two mice strains that can be useful for the study of the development of new therapeutic approaches.

Key words*: Borrelia burgdorferi, intracerebral, intradermal, neuroborreliosis, mouse.*

INTRODUCTION

Borreliosis or Lyme disease is caused by spirochetes belonging to the genus *Borrelia burgdorferi* (B.b.): B.b. sensu strictu, *B. garinii* and *B. afzelii* (Baranton, 1992). They are transmitted to humans and animals via ticks of the genus Ixodes (Zawada, 2020).

The history of borreliosis dates back to 1977, when it was clinically described as an infectious disease (Steere, 1977). The characteristic feature of the disease is a rash called erythema migrans complemented by signs of influenza, arthritis, cardiac or neurological disorders (van Dam, 1993; Shapiro, 2020).

The natural reservoir of B.b. is the white-footed mouse *Peromyscus leucopus* (Zawada, 2020).

In the process of transmitting borrelia into the human or animal body, changes in spirochete gene expression occur whereby they adapt and replicate. The motility and chemotaxis characteristics of borrelia enable systemic dissemination of infection by adhesion to the vascular wall or extravascular tissue, and the immune response generated determines the clinical expression of the disease. In humans, Lyme disease can persist for a long time before diagnosis and treatment, and in experimental infections in mice, borreliosis can persist for life (Rudolf, 2021).

The mouse model is often used to investigate the pathophysiology of human disease, including the study of Lyme disease. C3H/HN mice, have been shown to be most susceptible to borrelia infection, expressed clinically by inflammatory carditis and arthritic manifestations (Barthold, 1993). In these animals, the mode of induction of the disease that allows survival but especially migration and multiplication of spirochetes is intradermal inoculation, by this approach also

reproducing the natural conditions of infection (Kern, 2015). The skin is the organ that plays an essential role in the biology of borreliosis. At this level, borrelia cause chronic infection and from this point, migrate to other organs (Barthold, 1993).

On the other hand, there are reported data in the literature showing that borrelia could be recovered from the skin of mice almost a year after infection (Grillon, 2017). Several routes of disease induction have been tried over time (intraperitoneal, intradermal, intracerebral, subcutaneous, intravenous or by direct tick-animal contact) and it has been concluded that mice express disease when inoculated intradermally (Barthold, 1991). The strain of mouse appears to influence how the disease manifests, thus C3H mice develop severe joint and heart disease, C57Bl6 and BALB/c, mild forms of the same disease, and in SCID mice, the disease is highly aggressive, with arthritis and carditis reaching a maximum severity within 60 days of inoculation (Barthold, 1992).

Although the murine model is successfully used to study arthritis and carditis following Lyme disease, there is limited data in the literature focusing on the kinetics of B.b. in the nervous system (Garcia-Monco, 2013). For this reason, the aim of our study was to develop a mouse model of neuroborreliosis that would contribute to our understanding of host-pathogen interactions in the central nervous system (CNS).

MATERIALS AND METHODS

Ethics statement

The animal studies were approved by the Ethics Committee of the "Cantacuzino" National Institute for Medico-Military Research and Development (CI) and by the competent authority (Sanitary Veterinary and Food Safety Directorate Bucharest). The experiments were also carried out in accordance with EU Directive 63/2010 on the protection of animals used for scientific purposes.

Animals

Two mouse strains were used: BALB/c and CD1, derived from the CI Specified Pathogen-Free Animal Facility. Selection of animals at the start of the experiments was based on age (8 weeks), sex (females) and weight (approx. 22 grams). Mice were housed at the Experimental Medicine and Translational Research Platform facility in individually ventilated cages with 12-hour light-dark cycles. Animals were housed in groups of 5 (experiment no. 1) and 6 (experiment no. 2), respectively, and received water and food *ad libitum.*

Borrelia burgdorferi strain

The B.b. strain was provided by the Institute of Neuroimmunology of Kosice, Slovakia, and was isolated from the cerebrospinal fluid of a human patient. The strain processed by introducing a Green Fluorescence Protein (GFP) plasmid and a gentamicin-resistant gene. B.b. revitalisation was performed in the CI microbiology laboratory and for each experiment a low passage number (<6) Borrelia GFP strain was used, which was grown in BSK-H medium supplemented with 6% rabbit serum and 100 µg/1000 µl Gentamycin until the spirochete concentration was sufficient for use in the experimental infection. Inoculum was measured using the counting chamber of the phase contrast microscope and the concentration of borrelia used was set at 10^6 spirochaetes/mL.

Mice inoculation

Two studies were conducted using different strains of mice, different inoculation methods and different times to follow the disease progression as shown in Table 1.

Table 1. Outlining the study design

Experiment no. 1				
Strain	No of animals	Inoculation way	Monitoring period	Euthanized animals
BALB/c	5	intradermally	6 weeks	1 mouse BALB/c+ 1 mouse CD1
			8 weeks	2 mice BALB/c+ 2mice CD1
CD1	5		13 weeks	2 mice BALB/c+ 2mice CD1
Experiment no.2				
BALB/c	12	intradermally	1 week	3 mice ID+3 mice IC
		intracerebral	2 weeks	3 mice ID+3 mice IC

For each experiment, animals were anaesthetized with a mixture of ketamine (65 mg/kg, Vetased, Farmavet, Bucharest, Romania) and xylazine (5 mg/kg, Xylazine Bio 2%, Bioveta, Cluj-Napoca, Romania). The cervical and toraco-dorsal fur was trimmed and disinfected with 3% Iodine. Inoculation of spirochetes was performed in a laminar flow hood. For intradermal inoculation, a volume of 100 µL of borrelia medium, introduced as an intradermal bleb, was used in 10 different points, with orientation as close as possible to the cervical area. Intracerebral inoculation was performed by introducing a 10 µL volume of borrelia, using the same inoculation method described in another study of ours that involved injecting bacteria intra cisterna magna (Coman, 2023). After inoculation, animals were placed in clean cages and maintained in a noise-free environment to recover from anesthesia. At the end of each monitoring period, animals were euthanized by anesthetic overdose. Samples of dura mater, brain, bladder, ear, knee joint, and heart were collected from intradermally inoculated animals, and only dura mater and brain were collected from intracerebrally inoculated animals (Figure 1). Each organ was cut into smaller pieces and deposited in tubes with BSK-H culture medium, then incubated at 33ºC (Figure 2).

The tubes were examined macroscopically daily for signs of contamination.

Microscopic examination under fluorescence microscopy was performed twice weekly over a 6-week incubation period. For each positive tube, the number of incubation days until growth detection, growth quantification and other culture characteristics were noted.

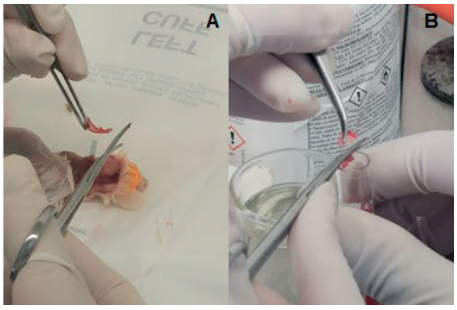

Figure 1. Harvesting of dura mater samples (A) and its fragmentation for deposition in culture medium (B)

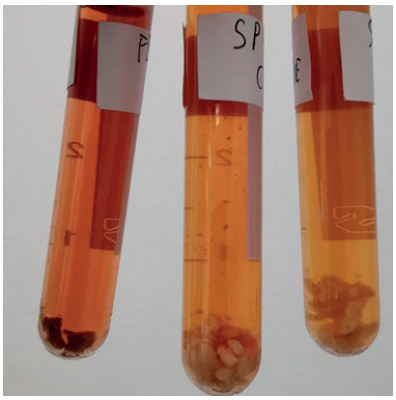

Figure 2. Brain, dura mater and organ samples harvested on BSK-H medium

RESULTS AND DISCUSSIONS

In terms of clinical signs, regardless of the route of inoculation or strain used, we did not observe any obvious symptoms. Two BALB/c mice showed a heavy gait, lameness on a hind limb, 4 weeks after intradermal inoculation, but these symptoms recovered 6 weeks after infection.

Following infection of the mice by intradermal inoculation, in the first experiment, we identified spirochete growth in tubes with medium containing ear samples in BALB/c animals and ear, bladder and knee joint samples in CD1, at the time of sample collection 6 weeks after infection. Growth in the tubes could be observed 20 days after sample collection.

Related to the analysis of samples collected at 8 and 13 weeks, no growth was observed in the tubes, even after 2 months of incubation (maximum follow-up period was 3 months). The number of animals involved in our first experiment is minimal, unlike other studies that used more animals that were followed for longer. Thus, if in the case of our experiment, the *Borrelia* showed an increase in the tube at 20 days after harvesting and no increase at the other times we can hypothesize that the surveillance interval is small compared to Barthold's study when he was able to isolate spirochetes even at 360 days after harvesting (Barthold, 1993) or even that the detection method approached did not detect spirochetes possibly due to the sensitivity limitation and the very small number of spirochetes (Table 2).

Table 2. The results of the first experiment cultural examinations

Samples	6 weeks		8 weeks				13 weeks			
	CD1	BALB/c	CD1	BALB/c	CD1	BALB/c	CD1	BALB/c	CD1	BALB/c
Dura mater	-	-	-	x	-	-	-	-	-	-
Brain	-	-	-	-	-	-	-	-	-	-
Ear	+	+	-	-	-	-	-	-	-	-
Bladder	+	-	-	-	-	-	-	-	-	-
Heart	-	-	-	-	-	-	-	-	-	-
Joint	+	-	-	-	-	-	-	-	-	-
+ (growth of spirochetes); - (no growth of spirochetes); x (contaminated sample)										

Previous studies have shown that the dissemination of B.b. and the severity of disease expression in laboratory mice is dependent on the route of inoculation. Therefore, in the second experiment we wanted to compare the intradermal and intracerebral route of inoculation, the aim being to identify the most efficient method by which borrelia reach the brain and express disease. Thus, the results showed that, one week after inoculation, 2 mice from each intracerebrally or intradermally inoculated group had borrelia in the dura mater samples collected. In animals sacrificed at 2 weeks post-inoculation, 3/3 intracerebrally inoculated animals showed borrelia in the dura mater, the increase being observed after 9 and 15 days of incubation, and in intradermally injected mice, no animal had B.b. positive samples (Table 3).

Table 3. The results of the second experiment cultural examinations

Samples	Intracerebral inoculation					
	1 week			2 weeks		
	Mouse no. 1	Mouse no. 2	Mouse no. 3	Mouse no. 1	Mouse no. 2	Mouse no. 3
Dura mater	+	X	+	+	+	+
Brain	-	-	-	-	-	-
Samples	Intradermally inoculation					
	1 week			2 weeks		
	Mouse no. 1	Mouse no. 2	Mouse no. 3	Mouse no. 1	Mouse no. 2	Mouse no. 3
Dura mater	+	+	-	-	-	-
Brain	-	-	-	-	-	-
Ear	+	-	-	-	x	-
Bladder	-	-	-	-	-	-
Heart	-	-	-	-	-	x
Joint	-	-	-	x	-	-
+ (growth of spirochetes); - (no growth of spirochetes); x (contaminated sample)						

As in an experiment by Divan et al., *Borrelia* were found in the dura mater at both 7 days and 14 days post intracerebral inoculation, in contrast to intradermal inoculation where we isolated spirochetes at 7 days but not at 14 days showing similarity to results reported during late disseminated infection (Divan, 2018).

Spirochete infection can disseminate systemically, reaching many tissues including the CNS where it produces lymphocytic meningitis, cranial neuritis or radiculoneuritis and facial paralysis (Ford, 2021; Eckman, 2018). On our mouse model, signs of nerve damage were not observed although through cultural examinations we were able to identify borrelia in the dura mater. This conclusion may correlate with the situation seen in humans, where approximately 15% of patients infected with B.b. show long-term neurological and psychological symptoms that do not respond to antibiotics (Rauer, 2020).

CNS damage occurs in many infections and often produces irreversible neurological damage (John, 2015). Spirochetes present in the CNS are important indicators for the onset of neuroinflammation (Koedel, 2015). As in our studies we identified borrelia in the brain in the first and second week after inoculation, we can

say that as time goes by, the infection enters a latent phase, so that at 6 weeks post-inoculation we found it in the tissues (experiment number 1), and at 8 and 13 weeks, most likely the spirochete titer decreased below the detection limit by the method of culturing the collected samples.

CONCLUSIONS

In these studies, we were able to develop neuroboreliosis through the inoculation of B.b. on two strains of mice, CD1 and BALB/c, with analysis of tissue samples collected at different time intervals demonstrating the systemic spreading action of *Borrelia*. Also, the intradermal and intracerebral inoculation route of B.b. in the two mouse strains were shown to be able to replicate the disease, which gives us a solid support for testing new drugs against Lyme disease.

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MISCELLANEOUS

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DISTRIBUTION AND PREVALENCE OF BRUCELLOSIS OUTBREAKS IN EUROPE IN THE PERIOD 2002-2022

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Abstract

Contaguous animal diseases that cause huge losses in livestock production and affect national economies are regulated at international level by the World Organisation for Animal Health (WOAH) through the Terrestrial Animal Health Code. The Code lays down the rules for the listed animal diseases that must be reported to the WOAH and among these important diseases is Brucellosis. This is a contagious bacterial disease caused by several representatives from the Brucella family which are infectious for specific animal species, including the most common livestock animals like cattle, sheep and goats and swine. Moreover, Brucellosis has a high zoonotic potential and could infect humans as well. In order to trace and analyse the distribution of Brucella spp. in Europe over the span of a twenty-years period (2002-2022), we derived data from the ADIS (Animal Disease Information System) managed by the European Commission which showed that five European counties were infected with variations in the prevalence of Brucellosis among the reporting member states. Regarding the identified species, it can be noted that the following were found: the predominant species is Brucella melitensis, followed by Brucella suis, and lastly, Brucella abortus. For the purpose of explainig the measures for disease management and control of Brucellosis we examined the main documents from the relevant secondary legislation of the EU. For the studied period, it can be noted that the most outbreaks of brucellosis were registered on the territory of Italy - 21 outbreaks (Brucella melitensis) from March 2014 in sheep to December 2022 in goats, but in the other years it was also found in sheep and goats. For the same period with Brucella abortus, there were 18 outbreaks from March 2015 in cattle to June 2022 in cattle, but in 2020, they were found in sheep and goats. Brucella suis was not registered.

Key words*: animal health, brucellosis, veterinary legislation, disease management, disease surveillance.*

INTRODUCTION

Zoonotic diseases nowadays are successfully brought under control in most of the developed countries, but some of them continue to be a constant threat to public health, animal health, and local economies. One of the bacterial diseases with great zoonotic potential is brucellosis, caused by various representatives from the *Brucella* family that affect different animal species. Some of the infections caused by *Brucella* spp. in animals are mandatory to be reported to the World Organisation for Animal Health (WOAH) - brucellosis in cattle caused by *B. abortus*, infection with *B. melitensis* in sheep and goats, and brucellosis in swine caused by *B. suis* (WOAH, 2023). At the same time, the infection was documented in humans long ago (Young, 1983; D`Anastasio et al., 2011) and as Doosti & Dehkordi (2011) argued it was described for the first time two millenia ago and isolated by Bruce in the end of the 19th century. Brucellosis is also known as "undulant fever", "Mediterranean fever", or "Malta fever" (Berhanu & Pal, 2020). Today, brucellosis continues to be a global burden, causing a severe debilitating disease in people (Dean et al., 2012), of which the most vulnerable appear to be veterinarians, farmers, and abattoir workers. As no licensed vaccine against human brucellosis is available, Heidary et al. (2022) emphasised that the control of the zoonosis in humans had to rely on the control, surveillance, and prevention of the disease in animals. The implementation of measures for the management of brucellosis is crucial in regions with insufficient levels of veterinary care, inadequate health and hygiene practices and unsupported food safety requirements - like many developing countries from Africa, central Asia, Middle East and the Mediterranean - where the highest rate of incidences of brucellosis were reported (Qureshi et al., 2023).

Smirnova et al. (2013) reported that currently ten representatives from the *Brucella* family are identified, among which some infect domestic and farm animals like *Brucella abortus* in cattle, *Brucella suis* in swine, *Brucella ovis* in sheep,

Brucella melitensis in goats, *Brucella canis* in dogs, some infect pest animals like *Brucella neotomae* and *Brucella microti* in rodents, while others are contagious for marine animals like *Brucella ceti* in cetaceans and *Brucella pinnipedialis* in seals. Due to the fact that the disease is persistent in livestock, cases of human brucellosis are frequently registered - more than half a million per year in the late 1990s (WHO, 1997), with a significant rise estimated at 2.1 million of annual global incidence in humans (Laine et al., 2023).

The complexity of the disease poses the need for an integrated and interdisciplinary approach (Rahama. et al., 2023) and was used to illustrate the concept of "One Medicine", which further developed into the "One Health" approach with a thorough understanding and principles in zoonotic disease management (Moriyón et al., 2023). With this regard, Faraz et al. (2018) proposed frequent serological surveillance accompanied by screening both categories of humans and animals for acute form of the disease with focus on people from risk groups and animals subjected to international trade, as key epidemiological activities to allow timely diagnosis and control of brucellosis.

The objective of the research is to review and analyze the regulatory provisions related to the implementation of the surveillance of the disease and to study the epizootic situation in Europe in relation to brucellosis.

MATERIALS AND METHODS

As brucellosis falls among the diseases that are regulated at the global level, we analysed the existing legislative framework on animal health control with regard to contagious and zoonotic animal disease. For this purpose, we assessed official documents from international legislation (OIE Terrestrial Animal Health Code), European regulations, and national (Bulgarian) legal acts. The implemented programmes for prevention, control, surveillance, and eradication of brucellosis in domestic animals were evaluated. The number and distribution of the disease outbreaks in livestock for the study period were analysed based on the information derived from the database ADIS (Animal Disease Information System of the EU).

RESULTS AND DISCUSSIONS

A number of countries worldwide were successful in eradicating brucellosis in their livestock, such as Australia, for example (More et al., 2015), and some EU member states like Croatia and Spain, where the disease is almost eradicated, while in Southern Europe (Greece, Italy, Portugal), the infection among livestock and zoonotic cases in humans still alert the public health authorities and the veterinary services (Jamil et al., 2022). The same authors, however, reported a decreasing trend in the number of reported brucellosis in humans in 2020 when compared to 2016, and as the primary etiological agent, *B. melitensis* was confirmed, followed by *B. suis*.

With regard to the complexity needed to address the management of contagious animal diseases and to enhance further the protection of animal health, a new Regulation (EU) 2016/429 was published which entered into force later in 2021. This sole legislative act replaces several other European regulations with the aim to implement a uniform approach to transmissible animal disesases with a focus on trade with live animals and products from them, mandatory reporting on disease outbreaks and implementation of measures for disease control and eradication. The rules laid down by this new "Animal Health law" require profound analysis on long-term epidemiological data and scientific risk assessment (Rankova & Balieva, 2023).

According to the categorization defined in Commission Implementing Regulation (EU) 2018/1882 of 3 December 2018 on the application of certain disease prevention and control rules to categories of listed diseases and establishing a list of species and groups of species posing a considerable risk for the spread of those listed diseases, brucellosis is a disease that belongs to category B.

Subjected to control on international and European level, Brucellosis is defined as "category B+D+E disease", which according to Art. 1, item 2, 4 and 5 of Commission Implementing Regulation (EU) 2018/1882 means a listed disease which must be controlled in all Member States with the goal of eradicating it throughout the Union, as referred to in Article 9 (1)(b) of Regulation (EU) 2016/429; means a listed disease for which measures are needed to

prevent it from spreading on account of its entry into the Union or movements between Member States, as referred to in Article 9 (1) (d) of Regulation (EU) 2016/429; means a listed disease for which there is a need for surveillance within the Union, as referred to in Article 9 (1) (e) of Regulation (EU) 2016/429.

Commission Decision 2003/467/EC lists the Member States and regions thereof with tuberculosis-free status, brucellosis-free status, and EBL-free status in accordance with Directive 64/432/EEC. Those Member States and regions thereof should be duly listed in the Annexes to this Regulation.

Commission Decision 93/52/EEC sets out the list of Member States and regions thereof with official brucellosis-free (*B. melitensis*) status in accordance with Directive 91/68/EEC. Those Member States and regions thereof should be duly listed in the Annexes to this Regulation.

Commission Implementing Regulation (EU) 2021/620 of 15 April 2021 laying down rules for the application of Regulation (EU) 2016/429 of the European Parliament and of the Council as regards the approval of the disease-free and non-vaccination status of certain Member States or zones or compartments thereof as regards certain listed diseases and the approval of eradication programmes for those listed diseases cancel Decision (2003/467/EC) and Decision 93/52/EEC.

The Commission Implementing Regulation (EU) 2021/620 contains a list of free zones and zones with eradication programs. In order to achieve status, a number of conditions are defined in Art. 66-72 of Commission Delegated Regulation (EU) 2020/689 of December 17, 2019, supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council as regards rules for surveillance, eradication programmes, and disease-free status for certain listed and emerging diseases. Disease-free status based on eradication programmes is acquired according to Art. 71 of Commission Delegated Regulation (EU) 2020/689.

For mandatory eradication programmes for category B diseases, a final report with additional annual information is submitted every 6 years. This applies to tuberculosis, rabies, and brucellosis until the disease is eradicated in the territory of the Member States. Final reports on the implementation of eradication programmes are submitted in order to apply for disease-free status.

In accordance with Art. 31, paragraph 1 of Regulation (EU) 2016/429), Member States which are not free or for which there is no information that they are free from one or more of the listed diseases referred to in Article 9, paragraph 1, letter b) of the same regulation, in their entire territory or in zones or compartments thereof, introduce a programme to eradicate the relevant disease or to prove that they are free from the specified disease from the list. The programme is implemented in relation to animal populations affected by the disease in question and covers relevant parts of the territories of the Member States or relevant zones or compartments thereof ("compulsory eradication programme") and applies until the conditions for obtaining disease-free status for the relevant territory of the Member State or zone. Member States submit a draft of the mandatory eradication programme to the EC for approval. It is approved by an act of implementation of the Commission, and more specifically, the Commission Implementing Regulation (EU) 2021/620. Commission Delegated Regulation (EU) 2020/689 determines the specific requirements regarding individual diseases for obtaining disease-free status, on the basis of which relevant programmes for the eradication of diseases are developed.

Eradication programmes - for diseases in category B are mandatory, with the goal of eradicating the disease and obtaining the free status of zones or compartments. The duration, deadlines for presentation, approval, and reporting of the programmes are determined, which is implemented every year through reports with the results of the conducted supervision, etc. data that show the progress of programme implementation.

Delegated Regulation (EU) 2020/689 establishes criteria for the granting of disease-free status for Member States or zones or compartments thereof, and the requirements for the approval of eradication programmes for Member States or zones or compartments thereof.

Recognition of disease-free status is determined in Annex V, Part II, Chapter 1, Section 1 to Commission Delegated Regulation (EU) 2020/689 of 17 December 2019 supplementing

Regulation (EU) 2016/429 of the European Parliament and of the Council as regards rules for surveillance, eradication programmes, and disease-free status for certain listed and emerging diseases. It is achieved at the territory level - for the territory of the entire country or a zone of it (Rankova & Balieva, 2023).

In December 2021, the Republic of Bulgaria received a notice from the European Commission, General Directorate "Health and Food Safety", about the need for revision of „Programme for supervision and control of the brucellosis disease (*Brucella melitensis*) in sheep and goats in the Republic of Bulgaria in 2022-2024" to fulfil the requirements of Annex IV, Part I, Chapter 1, Section 1 "Granting of the status" and Section 2 "Maintenance of the status" of Delegated Regulation (EU) 2020/689 with regard to the conditions for granting and maintaining the status of a livestock facility free from infection with *Brucella abortus, B. melitensis,* and *B. suis* without vaccination. The change affected the sampling scheme by increasing the scope of the susceptible small ruminants (sheep and goats) and the number of samples for serological testing. Until that moment, the programme provided for a one-time serological examination of all sheep and goats over 6 months old, reared in a herd with less than 50 animals and a one-time serological examination of 25% of sheep and goats over 6 months old, reared in a herd with more than 50 animals.

Decision No. 156 of March 18, 2022, of the Council of Ministers approved a National Programme for the prevention, supervision, control, and eradication of animal diseases, including zoonoses, for the period 2022-2024, as a part of which is "Programme for supervision and control of the brucellosis disease (*Brucella melitensis*) in sheep and goats in the Republic of Bulgaria in 2022-2024". The mentioned programme contains a new scheme for the surveillance of the disease, namely: all non-castrated sheep and goats over 6 months of age, raised on the territory of the country, should be examined once serologically. The rules applicable to individual diseases are listed in separate delegated acts, with a difference depending on whether it is a free zone or the disease is endemic to that zone/country:

1. CATEGORY B - rules for compulsory eradication programmes status „disease-free" in accordance PART II of Regulation (EU) 2016/429 and Commission Delegated Regulation (EU) 2020/689 and rules for prevention and control in accordance PART III of Regulation (EU) 2016/429 and Commission Delegated Regulation (EU) 2020/687;
2. CATEGORY D - rules for movements between Member States in accordance PART IV of Regulation (EU) 2016/429 and Commission Delegated Regulation (EU) 2020/688; or entry into the Union in accordance PART V of Regulation (EU) 2016/429 and Commission Delegated Regulation (EU) 2020/692;
3. CATEGORY E - rules for surveillance, diagnostics, and case definitions in accordance PART II of Regulation (EU) 2016/429 and Commission Delegated Regulation (EU) 2020/689.

To summarise the relevant European and Bulgarian legislative provisions on measures for control and eradication of Brucellosis, an overview of the legal framework is shown in Table 1 below.

Table 1. Corresponding legal acts on disease management regarding Brucellosis provided by the European and Bulgarian legislation

№	European legislation	National legislation
1.	Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in the area of animal health (Animal Health Law) (OJ L 84, 31.3.2016)	Law on veterinary activities (Prom. SG 87/1 Nov 2005, amend. SG 71/11 Aug 2020) (BG)
2.	Commission Implementing Regulation (EU) 2018/1882 of December 3, 2018, on the application of certain disease prevention and control rules to categories of listed diseases and establishing a list of species and groups of species posing a considerable risk for the spread of those listed diseases (OJ L 308, 4.12.2018)	Decision No. 156 of March 18, 2022 of the Council of Ministers approving a National Programme for the prevention, supervision, control and eradication of animal diseases, including zoonoses for the period 2022-2024 (BG)

3.	Commission Delegated Regulation (EU) 2020/687 of December 17, 2019, supplementing Regulation (EU) 2016/429 of the European Parliament and the Council, as regards rules for the prevention and control of certain listed diseases (OJ L 174, 3.06.2020)	
4.	Commission Delegated Regulation (EU) 2020/688 of December 17, 2019, supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council, as regards animal health requirements for movements within the Union of terrestrial animals and hatching eggs (OJ L 174, 3.6.2020)	
5.	Commission Delegated Regulation (EU) 2020/689 of December 17, 2019, supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council as regards rules for surveillance, eradication programes, and disease-free status for certain listed and emerging diseases (OJ L 174, 3.6.2020)	
6.	Commission Delegated Regulation (EU) 2020/692 of January 30, 2020, supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council as regards rules for entry into the Union, and the movement and handling after entry of consignments of certain animals, germinal products and products of animal origin (OJ L 174, 3.6.2020)	
7.	Commission Implementing Regulation (EU) 2020/2002 of December 7, 2020, laying down rules for the application of Regulation (EU) 2016/429 of the European Parliament and of the Council with regard to Union notification and Union reporting of listed diseases, to formats and procedures for submission and reporting of Union surveillance programes and of eradication programes and for application for recognition of disease-free status, and to the computerised information system (OJ L 412, 8.12.2020)	Ordinance No. 23 of 14.12.2005 on the procedure and method for announcing and registering infectious animal diseases (Prom. SG 6/20 Jan 2006), which introduces the requirements of Council Directive 82/894/EEC of 21 December 1982 on the notification of animal diseases within the Community (OJ L 378, 31.12.1982) (BG)
8.	Commission Implementing Regulation (EU) 2021/620 of April 15, 2021, laying down rules for the application of Regulation (EU) 2016/429 of the European Parliament and of the Council as regards the approval of the disease-free and non-vaccination status of certain Member States or zones or compartments thereof as regards certain listed diseases and the approval of eradication programes for those listed diseases *(*OJ L 131, 16.4.2021*)*	

Despite the strict legislative framework and all the preventive measures introduced at the international level, a lot of countries in the Mediterranean region and the Middle East remain endemic for brucellosis (Musallam et al., 2016). Control and eradication of this disease are dependent on its rapid detection and monitoring, but Alamiana et al. (2017) pointed out that the usual bacteriological methods were time-consuming and required special equipment and conditions for the detection of *Brucella* strains. At the same time the authors argued that there was a high similarity, among species within the *Brucella* genus, thus hindering their identification. Bahmani & Bahmani (2022) proposed a "test-and-slaughter" technique, introduced as a routine measure in Middle Eastern countries in order to eliminate the pathogen, combined with extensive livestock vaccination programmes.

In Italy, where brucellosis in small ruminants is still present, attention was paid to the importance of maintaining a proper protocol, including notifying the authorities about clinical symptoms such as abortions on farms, followed by epidemiological examinations for the confirmation of the infections and consequent implementation of the precautionary measures (Brunetti et al., 2023). This approach is also introduced through Regulation (EU) 2016/429

that determines measures against contagious animal diseases, a fundamental prerequisite for which is the maintenance of efficient information systems with reliable epidemiological data as ADIS.

Our analysis showed that for the study period (2002-2022), the prevalence and number of brucellosis outbreaks registered in the ADIS database varied among the EU member states. Regarding the identified species, it can be noted that the following were found: the predominant species was *Brucella melitensis*, followed by *Brucella suis*, and lastly, *Brucella abortus*. Similar findings were reported by De Massis et al. (2015) for Italy after the predominantly isolation of *B. melitensis* from farm animals from 156 outbreaks in 2011.

In September 2018, the first outbreak of the disease in sheep with *Brucella melitensis* was registered once in the Kingdom of Spain, while in Italy it appeared in January 2014 also in sheep, where 6 outbreaks were detected for the year. In 2015, the disease continued to be present, with the number of outbreaks increasing by another 10 per unit, but the trend was interrupted in 2016, as outbreaks were reported only for 2 units. In 2017, there were no confirmed cases of the disease, and since then only sporadic outbreaks have been registered (ADIS, 2023).

In the Kingdom of Spain, the peak of confirmed outbreaks was in 2021, when their number reached 9 out of a total of 14 for the study period.

For the study period (2002-2022), cases of brucellosis caused by *Brucella melitensis* were reported by 5 EU members states (Figure 1) - Austria (3 outbreaks during 2018 and March 2019 in cattle only), Cyprus (3 outbreaks during 2022 in cattle and sheep/goats (mixed herd), France (1 outbreak during November 2021 in cattle), Italy (21 outbreaks), and Spain (14 outbreaks during September 2018 in sheep to July 2021 in sheep, which is found mostly in sheep but is found in goats and cattle). The affected species of animals differ from country to country and can be divided into two main groups:

- ✓ cattle - Austria, Cyprus, and France;
- ✓ sheep and goats - Italy and Spain.

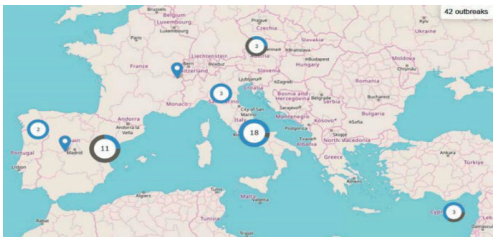

Figure 1. Reported outbreaks caused by *Brucella melitensis* in European member states in 2002-2022 (source ADIS)

For the study period (2002-2022), brucellosis (*Brucella suis*) outbreaks were declared in 4 countries in Europe (Figure 2) – France (2 outbreaks during February 2014 in cattle), Belgium (1 outbreak during December 2016), Spain (1 outbreak during October 2022 in sheep), and Portugal (1 outbreak during December 2022 in cattle). The affected animal species in all three countries were cattle, but in Spain in October 2022, the disease was detected in sheep.

Due to the fact that wildlife was also a part of the epidemiology of brucellosis and wild boars continued to serve as reservoirs for the pathogen, Lo Re III et al. (2024) argued that the incidence and geographic range of swine brucellosis will continue to expand.

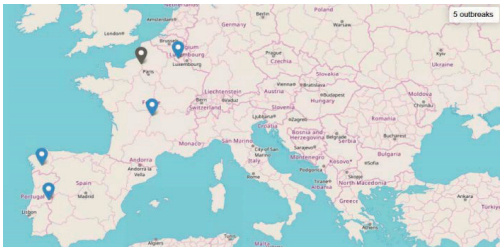

Figure 2. Reported outbreaks caused by *Brucella suis* in European member states in 2002-2022 (source ADIS)

For the study period (2002-2022), brucellosis (*Brucella abortus*) outbreaks were declared in 3 countries in Europe (Figure 3) - Italy (17 outbreaks), Spain (1 outbreak during August 2020), and Belgium (1 outbreak during December 2013). The affected animal species in all three countries were cattle, but in Italy in March 2020, the disease was detected in small ruminants.

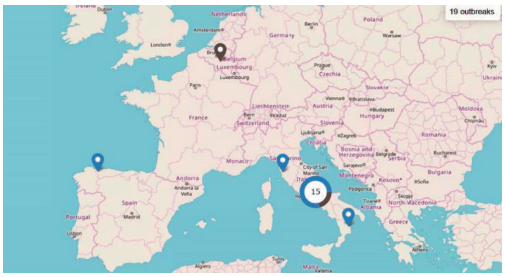

Figure 3. Reported outbreaks caused by *Brucella abortus* in European member states in 2002-2022 (source ADIS)

For the studied period, it can be noted that the most outbreaks of brucellosis were registered on the territory of Italy - 21 outbreaks (*Brucella melitensis*) during March 2014 in sheep to December 2022 in goats, but in the other years it was also found in sheep and goats. For the same period with *Brucella abortus*, 18 outbreaks were reported during March 2015 in cattle to June 2022 in cattle, but during 2020 it was found in sheep and goats. *Brucella suis*, was not registered.

The Kingdom of Spain was another of the notifying countries in which outbreaks of the disease with different representatives of the *Brucella* family (1 outbreak with *Brucella suis*) and (1 outbreak with *Brucella abortus*) were registered.

As the occurrence and reoccurrence of brucellosis are to some extent dependent on the characteristics of the communities such as level of education, economic development and cultural practices, there are a number of studies that confirm the crucial need for close cooperation between all stakeholders, starting from farmers to veterinarians, governmental bodies, policymakers, and international organisations (Khoshnood et al., 2022; Mohan et al., 2017). Moreover, for the success of the eradication programmes it is important to educate society (Pal et al., 2020), in particular animal owners, about brucellosis and its economic losses after an outbreak (Montaseri et al., 2024), as well as the zoonotic potential among high-risk groups (shepherds, butchers, and farmers) (Patel et al., 2023).

Further enhancement of the programmes related to the control of brucellosis could be achieved through the implementation of machine learning techniques to create algorithms for rhe prediction of the epidemiology of the disease. Thus, effective early warning systems and risk management could be executed by the public sector to minimise the impact of brucellosis on public health (Tito et al., 2023).

CONCLUSIONS

Regardless of the in-depth study of the causes and factors for the spread of brucellosis, it is still found everywhere in the world, including in the developed countries of the European Union. Its zoonotic nature gives it even greater importance, alongside economic importance, in the context of agricultural losses in the presence of an outbreak. That is why humane and veterinary doctors should not rule it out when making their diagnoses, since the control and prevention measures defined in European and national regulations are not sufficient to combat it, and a complex approach with vaccination programmes has to be conducted.

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Commission Delegated Regulation (EU) 2020/689 of 17 December 2019 supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council as regards rules for surveillance, eradication programes, and disease-free status for certain listed and emerging diseases (OJ L 174, 3.6.2020)

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