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Faculty of Veterinary Medicine

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ANATOMICAL PARTICULARITIES OF THE COCCYX IN OSTRICH (*STRUTHIO CAMELUS*)

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Abstract

This study, made on adult ostrich specimens, has the purpose of identifying the main characteristics of the pelvic belt at this bird and was motivated by the desire to complete existing data and to present anatomic elements using the terminology recommended by the Nomina Anatomica Avium. A very strong connection has been noticed between the ilium and the sacrum, strengthened by the forging in the medial plane of the preacetabular parts of the ilium. This aspect is very different from the ischium, which turns from a wide bone at the rest of the species, into a strong long bone at the ostrich that, with the exception of the cranial extremity, is separated through a wide space from the ilium. The absence of well-known anatomical features of birds from other orders was noticed, such as : the ilio-caudal fossa, the iliac oblique crest, the infracristal cavity, etc while observing the existence of some specific features like an unique type of pubic symphysis.

Key words*: ostrich, cooccyx, antitrochanter, pubis.*

INTRODUCTION

This species, by the scientific name of *Struthio camelus*, is the biggest living bird. It is valuable for feather production, egg production, as well for its skin and red meat with a very low fat content. (5). The domestic ostrich *(Struthio camelus domesticus)* is the result of over 100 years of selective cross-breedings, in the arid regions of South Africa, conducted with the purpose of improving this species' economical features. Although it does present some common characteristics of the locomotory system of other bird species (2, 6), the ratite have a series of anatomical differences determined by the loss of flight capacity but also by their size, being the largest representatives of their class.

In specialty literature, data regarding the skeletal system in ostrich is relatively brief (1,4,5) and doesn't include the recommended terminology of the Nomina Anatomica Avium (1,4,7,8), reason for which we have conducted a detailed study of the pelvic girdle at this species, seeking to complete the knowledge regarding the anatomy of this species.

MATERIALS AND METHODS

The study material was represented by parts from 10 adult specimens of different sexes, with weights between 70 and 85 kg, some corpses coming from zoos or private owners, brought to the Faculty of Veterinary Medicine of Bucharest in view of necropsy. Other specimens were provided from slaughterhouses. The bones were prepared through classic methods (maceration, manual cleaning, washing, degreasing, whitening and drying) then measurements were made, as well as describing the features and acquiring photographs. The identification, description and the certification of the formations were carried out according to the Nomina Anatomica Avium – 1993.

RESULTS AND DISCUSSIONS

At the ostrich, the ilium is a massive bone, composed of a shorter and wider preaceabular part (*Ala preacetabularis ilii*) and a very long and almost completely independent of the ischium, postacetabular part (*Ala postacetabularis ilii*) [Fig. 1(1,2)]. The ratio between the length of the preacetabular part and

the postacetabular part (taking as a landmark a vertical plane that crosses through the center of the acetabular hole) is 1 : 2.

On the dorsal side (*Facies dorsalis*), the dorsal iliac fossa (*Fossa iliaca dorsalis*) has the appeareance of an almost plain surface, strongly ventrally inclined and marked for the most part by lines and coarse crests.

The dorsal iliac crest (*Crista iliaca dorsalis*) [Fig.1(5)], rectilinear in its middle third, is united with the one on the opposite side, forming a median elongated relief that becomes progressively thicker at the cranial extremity. In the caudal part, the dorsal iliac crest will separate from her symmetrical one, recurving laterally to end through a prominence oriented towards the dorsal margin of the antitrochanter from which it is separated by a large incisure.

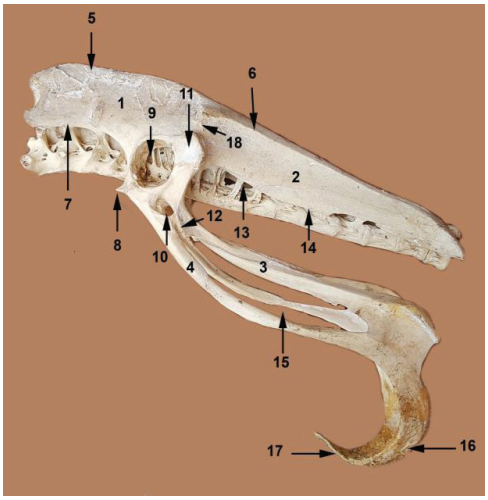

Fig. 1. The coccyx of the ostrich – lateral view (original)
1-the preacetabular part of the ilium; 2-the postacetabular part of the ilium; 3-ischium; 4-pubis; 5-the dorsal iliac crest; 6-the dorso-lateral crest of ilium ; 70- the lateral iliac crest ; 8-the preacetabular tuber (pectineus) ; 9-the acetabular hole; 10-the obturator hole; 11-the antitrochanter; 12-the obturator process; 13-the transverse processes of the sinsacrum's vertebrae; 14-the infracristal blade; 15- the ischio-pubic hole; 16-scapus pubis; 17-the apex of the pubis

The lateral iliac crest (*Crista iliaca lateralis*) [Fig.1(7)] is convex in the cranial half and concave in the caudal one, oriented in ventro-lateral direction. At a small distance from the cranial margin of the acetabular hole it extends ventrally reaching the preacetabular tuber (pectineal process) [Fig.1(8)].

The plain ventral face (*Facies ventralis*) is ventro-medially oriented towards the spinous or dorsal crest of the sinsacrum (*Crista spinosa synsacri*).

The sharp angle that forms with the median plan enables us to consider, from a topographic point of view, this face as a medial face rather than a ventral one.

Apart from this, between the ventral face and the spinous crest a wide space can be observed, with an almost triangular outline in a transversal section.

In the inferior part, this space is delimitated by the transverse processes of the last two thoracic vertebrae and the first three-four lumbar vertebrae that, through their apex, articulate with the ventro-median margin of this face while leaving very large spaces in the area of the intertransversary openings (*Fenestrae intertransversarie*) [Fig. 3(2)] at this species.

At young birds, where the joints between the ilium and the top of the transverse processes did not weld, the articular areas (*Arae articulares vertebrales*) that correspond to these processes can be observed.

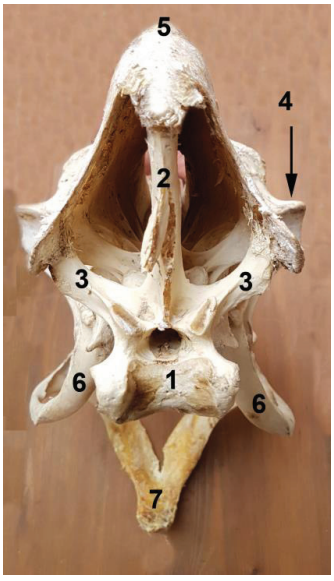

Fig. 2. The coccyx of the ostrich- cranial view (original)
1-the cranial terminal face of the second last thoracic vertebrae ; 2-the thorny crest of the sinsacrum; 3-the transverse processes of the second last lumbar vertebrae; 4-the antitrochanter; 5- the dorsal iliac crests united in a medial plan; 6-pubis; 7-the pubic symphysis.

The postacetabular part of the ilium also has three faces: a dorsal, a lateral and a ventral one.

Because of the totally different appearance of the coccyx in these ratite, the terms for these faces don't fully correspond to the topographic reality.

The dorsal face (*Facies dorsalis*) is separated by the lateral face through the dorso-lateral crest of the ilium (*Crista dorsolateralis ilii*) (indistinct at some anseriformes, for example).

The dorso-lateral crest is long, almost rectilinear (with the exception of the extremities) and is more prominent in the cranial half than the caudal one.

In the caudal part, the caudal crest thickens, becomes coarse and slightly orients ventro-laterally, ending through a sharp process that matches the dorso-lateral spina of the ilium (*Spina dorsolateralis ilii*).

Cranially, the crest is oriented latero-ventrally, connecting to the caudal extremity of the dorsal iliac crest, at the level of the tuber that is oriented towards the antetrochanter, a process reminded earlier that is not mentioned in specialty nomenclature.

The dorsal side of the postacetabular part is very long, transversally convex on its entire length.

A genuine iliocaudal fossa (*Fossa iliocaudalis*) is not identified. At the species at which it exists, this fossa is destined to the insertion the *levator caudal* muscle.

Taking into account the role of the tail in directing flight, the absence of this fossa in the ostrich would be due to the weak development of the tail musculature in ratite, birds without the capacity of flight.

Unlike the preacetabular part, the medial margin of this dorsal face is not in contact with the symmetric one and for most of its part it also remains separated from the sinsacrum through a large space.

The ilio-sacral suture (*Suture iliosynsacrala*) is present just in the posterior third of the bone.

The lateral face (*Facies lateralis*) of the postacetabular portion is vaster compared to the dorsal one. The ventral margin of the bone blade that constitutes this face (*Lamina infracristalis ilii*) is loose on its entire length and there is no ilio-ischiatic synchondrosis (*Syncondrosis ilioischiatica*) therefore the ilioischiatic hole doesn't form either (*Foramen ilioischiaticum*).

The infracristal cavity cannot be identified (*Cavitas infracristalis*), the reduced depression is situated caudally from the ilioischiatic hole and it is destined to the ischiofemuralis muscle.

However, this side is obliquely crossed in a cranio-caudal and ventro-lateral way by an obvious crest, not mentioned in literature.

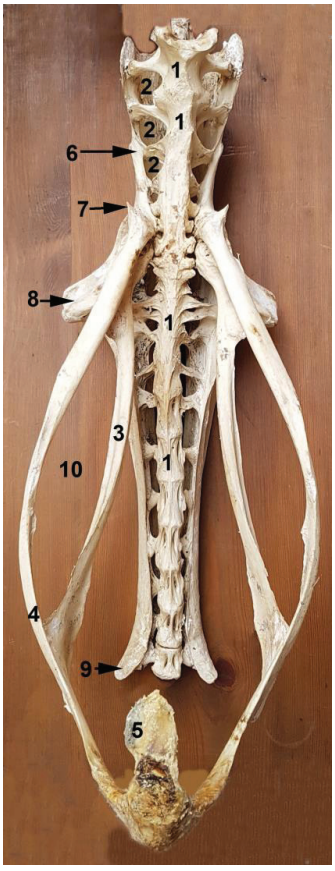

Fig. 3. the coccyx of the ostrich – ventral view (original)
1-sinsacrum; 2-the intertransversal openings; 3-ischium; 4-pubis; 5-the apex of the pubis; 6-the lateral crest of the ilium; 7-the prepubic tuber; 8-the antitrochanter; 9-the dorso-lateral spina of the ilium; 10-ischio-pubic hole.

The ventral side of the postacetabular part orients itself in a medial direction and is articulated with the transverse processes of the vertebrae that compose the sinsacrum [Fig. 3(1)].

The caudal crest of the renal pits (*Crista caudalis fossae renalis*) is indistinguishable and so is the caudal recess of the pits (*Recessus caudalis fossae*).
The body of the ilium (*Corpus ilii*) represents the central part of the ilium, situated dorsally form the acetabular cavity. A formation corresponding to the oblic iliac crest (*Crista iliaca obliqua*) of other species could not be identified. The antitrochanter (*Antitrochanter*) [Fig. 3(8)] is prominent; it has an articular surface (*Facies articularis femoralis*) with a rectangular look and is well-defined dorsally by a wide antitrochanteric groove (*Sulcus antitrochantericus*). The preacetabular tuber (*Tuberculum preacetabulare (Processus pectinealis*) [Fig. 1(8)] is placed on the ventro-cranial margin of the acetabulum, and it is represented by a short and sharp spina. The acetabular hole (*Acetabulum*) [Fig. 1(9)] is large, with a circular contour and an approximate diameter of 65 mm.
The ischium [Fig. 3(3)] is considerably different from other species, being long and very narrow. The anterior extremity, which matches the body of the ischium (*Corpus ischii*) participates in the formation of the acetabular cavity and, through the ventral margin, at the formation of the foramen obturatum (*Foramen obturatum*) [Fig. 1(10)]. This hole has an ellipsoidal outline and estimated sizes of 20/30 mm in adults. It is caudally delimited by a short, obtruding process (*Procesus obturatorius*) articulated with the pubis on a length of 15 mm.
The rest of the ischium corresponds to the wing (*Ala ischii*) and is crossed on its entire surface by a prominent sharp crest.
Between the wing of the ischium and the postacetabular portion of the ilium there is no link, the margins of the two bones being separated on their entire length by a very large space.
The puboischiatic rift (*Fenestra ischipubica*) is very large and long and thus it articulates with the pubis just at its extremities.
The pubis is very long. Through the anterior extremity (*Corpus pubis*) it participates at the formation of the acetabular cavity.
The caudal portion (*Scapus pubis*) takes part in the delimitation of the puboischiatic rift, and it articulates with the caudal extremity of the ischium, caudally exceeding this extremity and recurving ventrally and slightly medially. The apex of every pubis (*Apex pubis*), unlike the rest of the domestic birds, welds with the symmetrical opposite forming a symphysis that is not found in any other birds species.

CONCLUSIONS

In contrast with other bird species, the ostrich's ilium is generally a massive bone, very well consolidated with the sinsacrum but still reduced in comparison to it.
It is a little extended laterally, forming, as a whole, a sharp angle with the vertical plane which makes the ceiling of the abdominal cavity appear relatively narrow.
As opposed to the rest of the bird species, in the ostrich, the dorsal iliac crest, rectilinear in its middle third, is united with the one from the opposite side, forming a median elongated relief, which progressively thickens at the cranial extremity.
At this species a genuine iliocaudal fossa is not identified for the insertion of the caudal levator muscle because it functionally correlates with a weak development of the tail of this species, the ostrich being a bird without flight capacity.
The ventral margin of the bone blade that forms the lateral face of the postacetabular portion of the ilium is loose along its entire length at distance from the ischium, an ilio-ischiatic synchondrosys being unidentifiable.
At the ostrich both the caudal crest of the renal pits and the caudal recess of the pits are indistinguishable. A process corresponding to the oblique iliac crest that is present on the ilium's body in other species could not be identified.
Compared with other bird species, in ostrich, the ischium is considerably different, being long and very narrow.
Unlike all domestic bird species, in the ostrich, the apex of every pubis welds with the symmetric forming a symphysis.

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ASPECTS REGARDING THE MORPHOLOGY OF CERVICAL VERTEBRAE IN COYPU (*MYOCASTOR COYPUS*)

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Abstract

This study was done on three bodies of adult coypu. The material was obtained by cleaning and macerating bones in 37°C water. Following the whitening process the bones were consequently cleaned under a water stream and left to dry. The cervical vertebrae generally have the spinous processes at a uniform height, oriented caudally for vertebrae III-V, dorsally for vertebra VI and cranially for vertebra VII. The body of the cervical vertebrae is short and flattened dorso-ventrally; the cranial and caudal terminal facets are plane. The ventral vertebral crest is absent. The transverse processes are the same length, slightly more developed at vertebra VII. The transverse foramen is present, wide in diameter, being replaced at vertebra VII by a vertebral incisura. The atlas has rounded transverse processes, oriented dorsally. The transverse foramen is located on the caudal edge of the atlas wing. The lateral vertebral foramen and the alar foramen are joined through a thick alar notch. The axis has a thick, developed spinous process that ends in a tuberosity. Its transverse processes slightly surpass the caudal terminal facet.

Key words*: cervical vertebrae, coypu, vertebral column.*

INTRODUCTION

Morphological aspects of the cervical vertebrae in coypu are important because they present certain particularities compared to other species of rodents (Coțofan et al., 1982, Hrițcu et al., 2000, Predoi, 2012). There are 7 cervical vertebrae, and the diverse aspects described in this study complete already existing data from scientific literature (Coțofan et al., 1987, Coțofan et al., 2003, Predoi et al., 2001).

The body of the cervical vertebrae is reduced, transversally wider and with a very large vertebral canal. The transverse foramen is wide, oval in shape and constantly located on the caudal edge of the atlas wings.

Unlike in scientific literature, where a cranial vertebra incisura is described for the atlas, this study revealed the presence of a large lateral vertebral foramen. The alar notch between the alar foramen and the lateral vertebral foramen is thick. Particularities of this species were also discovered in the transverse processes of vertebrae V and VI. In the seventh vertebra the transverse foramen is replaced with an incisura.

MATERIALS AND METHODS

Three bodies of adult coypu were used as material. The process of controlled maceration was used as method.

This particular method takes the following steps:

- Skinning the body
- Eviscerating the body
- Manually removing muscle mass from the bones.

The controlled maceration technique includes submerging the bones in water at 37°C. Following the maceration process the bones were washed under a stream of water and were submitted to a whitening process.

For the whitening process a solution of hydrogen peroxide 11% was used.

After the whitening process the bones were once again cleaned under a stream of water and left to dry at room temperature.

Following the preparation process, the bones were studied and photographed.

The naming of the structures was done in concordance with the norms imposed by Nomina Anatomica Veterinaria - 2005.

RESULTS AND DISCUSSIONS

In the cervical region, the vertebrae are characterised by a short body which is transversally wide. A wide vertebral canal can be observed. The atlas has well developed rounded wings that are slightly deviated dorsally (Fig. 1, 2, 3).

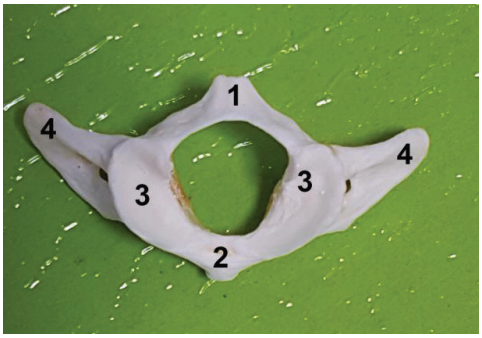

Fig. 1 Atlas of coypu, cranial view (original)
1-dorsal tubercle; 2-ventral arch; 3-glenoid cavities; 4-wings of the atlas.

The transverse foramen is large, oval shaped, placed horizontally on the caudal edge of the atlas wings.
Compared to scientific literature, this study reveals the bone to have a large lateral vertebral foramen as well as an alar foramen, united through an alar notch.
The cranial articular surfaces are slightly excavated.

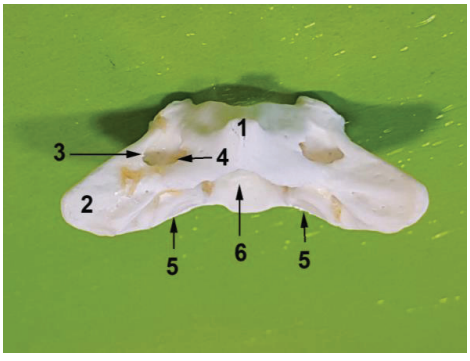

Fig. 2 Atlas of coypu, dorsal view (original)
1-dorsal tubercle; 2-wings of the atlas; 3-hole alar foramen; 4-transverse foramen; 5-caudal articular surfaces; 6-vertebral foramen.

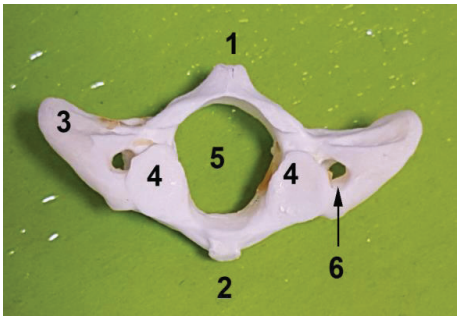

Fig. 3 Atlas of coypu, caudal view (original)
1-dorsal tubercle; 2-ventral arch; 3-wings of the atlas; 4-caudal articular surfaces; 5-vertebral foramen; 6-transverse foramen.

The axis has a well-developed spinous process, thick all throughout its length, which ends dorsally in a reduced tuberosity.
The transverse processes are developed and slightly surpass the terminal caudal facet. The transverse foramina are broad with vertebral incisurae which are also wide, while the terminal caudal facet has a shallow glenoid cavity.
The terminal cranial facet features an odontoid process in the shape of a cone that is slightly deviated dorsally.

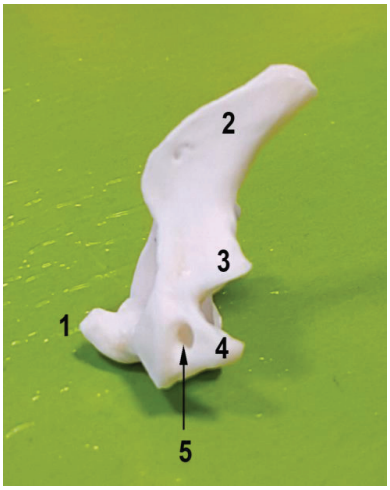

Fig. 4 Axis to coypu, lateral view (original)
1- odontoid process; 2-spinous process; 3-caudal articular processes; 4-transverse processes; 5-transverse foramen.

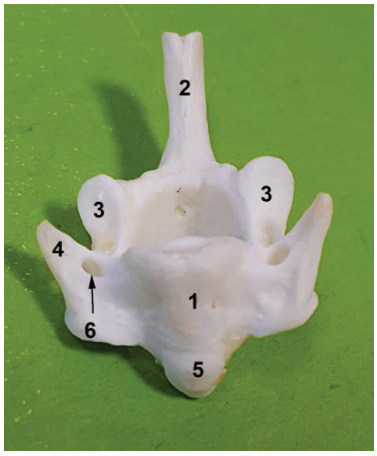

Fig. 5 Axis to coypu, ventro-caudal view (original)
1-the body of axis; 2-spinous process; 3-caudal articular processes; 4-transversale process; 5- odontoid process; 6-transversale foramen.

The cranial articular surfaces are triangular in aspect. The caudal articular processes emerge at a distance from the spinous process and appear flat (Fig. 4, 5).
The third cervical vertebra has a short, dorso-ventrally flattened body. The ventral vertebral crest is absent. Both the terminal cranial facet and the terminal caudal facet appear flat. The spinous process is relatively well developed, but it lacks a dorsal tuberosity. The transverse processes exceed the terminal caudal facet, oriented dorso-caudally. The transverse foramen is wide. (Fig. 6)

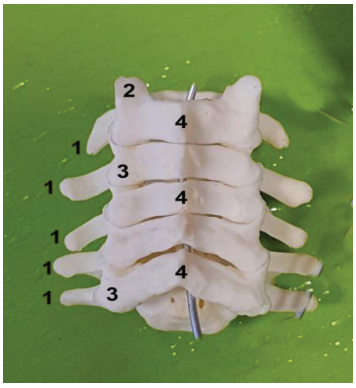

Fig. 6. Cervical vertebrae III-VII in coypu, dorsal view (original)
1-transverse processes; 2-cranial articular processes; 3-caudal articular processes; 4-spinous processes.

The fourth cervical vertebra exhibits wider and thicker transverse processes compared to the third vertebra, while the spinous process is dorsally widened and levelled with the one corresponding to the previous vertebra.
The fifth cervical vertebra has a widened transverse process, oriented in the same direction as in the case of the third and fourth vertebrae. The transverse foramen is wide.
Ventro-medially from the transverse process an osseous dint can be observed, which is flattened, oriented cranially and ended with a crest.
The sixth vertebra has a short body, flattened dorso-ventrally, and it exhibits flat terminal faces as well as the absence of the ventral vertebral foramen. The transverse foramen is very wide. The transverse processes feature ventro-caudally two thick osseous laminae which are well detached and flattened.

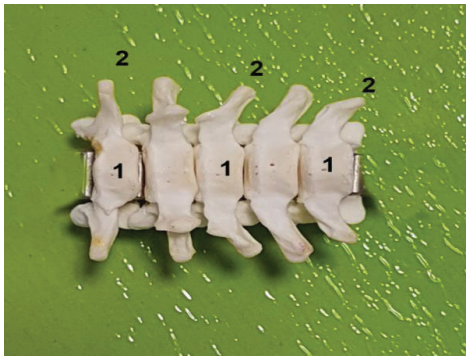

Fig. 7 Cervical vertebrae III-VII in coypu, ventral view (original)
1-vertebral body; 2-transverse processes;

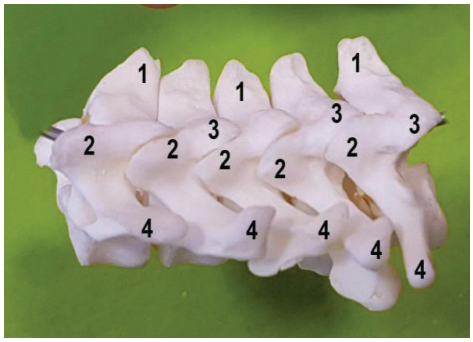

Fig. 8 Cervical vertebrae III-VII to coypu, lateral view (original)
1-spinous processes; 2-cranial articular processes; 3-caudal articular processes; 4-transverse processes.

The seventh cervical vertebra features its spinous process oriented cranially, widened at the same height as in the previous vertebrae.
The transverse process is very wide dorso-ventrally and is oriented caudally.
Instead of transverse foramina, vertebral incisurae are present (Fig. 7, 8).

CONCLUSIONS

The bodies of the cervical vertebrae is short and flattened dorso-ventrally, and it lacks a ventral vertebral crest.
The vertebral canal of this species is particularly wide.
Unlike what is presented in existing specialty literature, the atlas does present a wide lateral vertebral foramen and not a lateral vertebral incisura. The alar foramen is also present. The transverse foramen is on the caudal edge of the atlas wings.
The axis does present lateral vertebral incisurae, a cone-shaped odontoid process that is slightle deviated dorsally and the caudal terminal facet is represented by a shallow glenoid cavity.
The spinous process of the III-VII vertebrae is thick and levelled.
In the fifth vertebra, the transverse process features an osseous dint that is flattened cranially and ends with a crest.
In the sixth vertebra the transverse processes feature ventro-caudally two thick osseous laminae which are well detached and flattened.
The seventh vertebra features an incisura in place of the transverse foramina.

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CANINE HERPESVIRUS-1 SPECIFIC SEROCONVERSION AND CLINICAL ASPECTS IN KENNEL DOGS FROM ROMANIA

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Abstract

The Canine herpesvirus-1 (CHV-1) is causing in dogs a wide range of reproductive problems: infertility, foetal resorption, abortion, weak puppies, stillborn, low conception rate, small litter size and neonatal mortality, according to the age and pregnancy stage. The aims of the study where to assess the status of CHV-1 infection and to investigate the clinical pattern of the disease, in three Romanian kennel dogs. Blood samples from 44 subjects, aged from 1 to 5.5 years (20 dogs from kennel A, 16 dogs from kennel B, and 8 from kennel C), without history of vaccination against CHV-1 where submitted to study. The serum samples were analysed for the detection of antibodies to CHV-1 by immunofluorescence assays. In this survey, the average of seropositive animals were being 86.36%, but ranged from 100% in kennel A and B, to 25.00% in kennel C. Registered reproductive disorders were represented by neonatal mortality (70%) and infertility (30%). Our study emphasizes the widespread of CHV-1 infection and strengthens the recommendation for the animals' immune status assessment before their breeding season.

Key words*: CHV-1, immunofluorescence assay, canine infectious diseases, reproductive pathology.*

INTRODUCTION

Canine herpes virus infection (CHI) is an acute disease reported in dogs, wolves and coyotes, clinically characterized by respiratory, ocular and genital/reproductive disorders (Carmichael et al., 1965; Poste and King, 1971; Carmichael and Greene, 1998).

The first description of CHI was done by Carmichael et al. (1965) as a fatal septicaemia disease of puppies. Since then, numerous studies have been carried out, enabling the complete characterization of the etiological agent and the worldwide spread of Canine herpesvirus-1 (CHV-1) (Spertzel et al., 1965; Lundgren et Clapper, 1969; Huxtable and Farrow, 1970; Delisle, 1982; Takumi et al., 1990; Gaskell and Willoughby, 1999; Carmichael and Greene, 1998).

CHV-1 is a virus belonging to family *Herpesviridae*, subfamily. *Alphaherpesvirinae*, genus *Varicellovirus*. CHV-1, Feline herpes virus-1 (FVH-1) and Phocine herpesvirus-1 (PhHV-1) are closely related genetically (Gaskell and Willoughby, 1999). Most viruses range in size from 115 to 175 nanometres (nm). The virus is replicating in Dog Kidney Cells, producing cytopathic effect in 2-3 days (Spertzel et al., 1965; Carmichael and Greene, 1998).

The highest prevalence of CHI is obvious mainly in animal clusters without specific surveillance programs. It has been reported in the USA (Carmichael et al., 1965; Lundgren et Clapper, 1969), Europe (Delisle, 1982), Australia (Huxtable and Farrow, 1970), Asia and Oceania (Takumi et al., 1990).

CHV-1 can be transmitted horizontally through direct contact with infected material (e.g., uterine secretions, oronasal secretions) and transplacental (Hashimoto et al., 1982).

The infection is prevalent in many countries and produces significant losses due to reproductive pathology and neonatal death (Carmichael and Greene, 1998).

The reproductive pathology is represented by low conception rate, embryonic and foetal death followed by resorption or abortion or stillborn puppies and small litter size (Poste and King, 1971).

Also, CHV-1 is one of the etiological agents of the canine infectious respiratory disease complex, alongside several other canine viruses, such as canine adenovirus type 2, canine

respiratory coronavirus, canine influenza virus, and canine parainfluenza virus (Buonavoglia and Martella, 2007), as well as bacteria *Bordetella bronchiseptica, Streptococcus equi* subsp. *Zooepidemicus*, and *Mycoplasma cynos* (Zeugswetter et al., 2007; Priestnall et al., 2010; Singh et al., 2015).

Respiratory disorders are described in older dogs and the clinical signs are mild, usually restricted to the upper respiratory tract (e.g. nasal discharge, coughing); the pneumonia is rare (Appel et al., 1969).

Diagnosis of any suspicion of CHI, followed by correct management and a cautious attitude towards animals with clinical signs are crucial in the fight against this pathogen. These state of art make Ronsse et al. (2003) to conclude that "*A good collaboration between breeders, veterinarians and laboratories will allow a rapid intervention, able to limit the often considerable economic losses*" (Ronsse et al., 2003).

In Romania, CHI is underdiagnosed and frequently the symptomatology is associated with other causes.

Also, the confirmation diagnosis is not applied on a regular basis. This led to the lack of knowledge of the prevalence of CHV-1 infections in Romania.

Since dog breeding increased in Romania in the recent years then it has become an imperative requirement to confirm the status of breeding animals - kennel animals, versus CHV using appropriate diagnostic methods.

The primary objective of this research was to design a comprehensive protocol for the diagnosis of CHV-1 infection, according to the Romanian particularities.

The second objective was to identify among canines with reproductive disorders the ones who, based on the anamnesis provided by the owner and on the clinical signs expressed, match to the specific pattern of the CHV infection suspicion.

The third objective was to propose an easy, fast, highly specific and reasonably priced method, in order to confirm the diagnosis suspected/presumed. The last objective was to associate clinical symptomatology with the animal's immune status in relation to the presence of CHV-1 infection.

MATERIALS AND METHODS

For this survey, three Romanian breeding kennels were chosen, below identified as A, B and C. The size and structure of populations are listed as fallow.

Table 1. The size and structure of Romanian breeding kennels

Population	Kennel A	Kennel B	Kennel C	Total
Male	5	4	2	1
Female	15	12	6	33
Total	20	16	8	44

In total, investigated kennels owned 44 dogs (Table 1) of different ages, breeds, number of pregnancies, and performance in reproduction and without history of vaccination against CHV-1.

Blood samples from each subject, where collected from the cephalic vein, in vacutainer blood collection tubes without additives, Samples were stored in the refrigerator until centrifugation (15 min at 2,200 rpm) and the sera has been collected in sterile Eppendorf tubes (1.5 ml). The serum was stored at −20°C until serological testing.

The serum samples were analysed with a commercial immunofluorescence assay (IFA) designed to detect antibodies to CHV-1 (FluoHERPESVIRUS canine, Agrolabo, Italy). IFA method was performed as recommended by the manufacturer. Briefly, all reagents were brought to room temperature (20-25°C) before testing and each serum has been diluted 1:80 in buffered saline.

For each serum to be tested and for the Negative and Positive controls were used 20 µl in the individual slide wells, pre-coated with inactivated cells infected with CHV antigens. Incubation was done in humid chamber for 30 minutes at 37°C. The conjugate anti-Dog-IgG-FITC was added in each well, in the same volume (20 µl/well) and incubated in the same conditions in the dark. The lecture of stained substrate slides was performed at 400X magnification. The samples providing negative results at 1:80 screening dilution were considered negative for CHV-IgG antibodies, and the ones providing positive test results at 1:80 screening dilution were considered positive for CHV-IgG antibodies.

RESULTS AND DISCUSSIONS

In the first investigated kennel, the serological assessment of CHV-1 circulation was based on the history of the infertile mating and on the neonatal mortality (Table 1).

Table 1. Reproductive pathology associated with immune status in kennel A

No. #	Breed	Gender	IFA result	No of infertile matings	No of litters	Litter size	Neonatal mortality
1.	Rottweiler	F	+	1	1	4	2
2.	Rottweiler	F	+	0	0	0	0
3.	Rottweiler	F	+	0	1	4	4
4.	Rottweiler	F	+	0	1	4	0
5.	Rottweiler	F	+	0	1	5	0
6.	Rottweiler	F	+	0	1	4	0
7.	Rottweiler	F	+	0	1	5	2
8.	Rottweiler	F	+	1	0	0	0
9.	Rottweiler	F	+	0	0	0	0
10.	Rottweiler	F	+	0	1	6	0
11.	Rottweiler	F	+	0	1	5	5
12.	Rottweiler	F	+	0	0	0	0
13.	Rottweiler	F	+	0	1	5	5
14.	Rottweiler	F	+	1	0	0	0
15.	Rottweiler	F	+	0	1	7	2
16.	Rottweiler	M	+	-	-	-	-
17.	Rottweiler	M	+	-	-	-	-
18.	Rottweiler	M	+	-	-	-	-
19.	Rottweiler	M	+	-	-	-	-
20.	Rottweiler	M	+	-	-	-	-
TOTAL				**3**			**20**

In the kennel A, the reproductive disorders suddenly appeared, with several cases in a short period of time. The intensity of signs was different in affected animals: 100% neonatal mortality in bitches' litters #3, #11, and #13, while bitch #7 had 60.00% neonatal mortality, bitch #1 had 50.00%, bitch #15 had 28.57%, and bitches #4, #5, #6, and #10 had 0.00% neonatal mortality. Bitch #1 seems to have been the most affected, expressing both, infertile matings and neonatal mortality.

Overall, in the kennel A was 20 cases of neonatal mortality (death in first 72 hours of life), in a total of nine calving with 49 newborne puppies.

In second investigated kennel, the serological evaluation of CHV-1 circulation started after several multiple cases of neonatal mortality (Table 2).

Table 2. Reproductive pathology associated with immune status in kennel B

No. #	Breed	Gender	IFA result	No of infertile matings	No of litters	Litter size	Neonatal mortality
1.	Cane Corso	F	+	0	1	7	3
2.	Cane Corso	F	+	0	2	6	2
						8	1
3.	Cane Corso	F	+	0	2	10	3
						10	1
4.	American Staffordshire Terrier	F	+	0	2	7	2
						7	2
5.	American Staffordshire Terrier	F	+	1	1	6	6
6.	American Staffordshire Terrier	M	+	-	-	-	-
7.	American Staffordshire Terrier	M	+	-	-	-	-
8.	American Bully	F	+	0	0	0	0
9.	American Bully	F	+	0	0	0	0
10.	American Staffordshire Terrier	F	+	1	0	0	0
11.	American Bully	M	+	-	-	-	-
12.	American Staffordshire Terrier	F	+	0	2	10	2
						8	1
13.	Cane Corso	F	+	1	1	8	8
14.	Cane Corso	M	+	-	-	-	-
15.	Cane Corso	F	+	0	2	16	0
16.	American Staffordshire Terrier	F	+	0	1	5	5
TOTAL				**3**			**36**

As in the previous described kennel, in the kennel B, the main reproductive disorder was the neonatal mortality. The intensity of clinical signs registered variation from one case to another, with 100% neonatal mortality in litter of bitches #5, #13, and #16, 30.00% in litter of bitch #1, 28.57% in litter of bitch #4, 21.43% in litter of bitch #2, 20.00% in litter of bitch #3, 16.67% in litter of bitch #12, and 0% in litter of bitch #15.

Bitch #13 have been the most affected: she suffered one infertile mating and a calving with 100% neonatal mortality. It must be also emphasized the persistence of neonatal mortality in all bitches with two litters. In this kennel, all breeds (Cane Corso, American Staffordshire Terrier, and American Bully) were affected.

Generally, in the kennel B was 36 cases of neonatal mortality from the 13 calving, with 108 new-borne puppies.

In the kennel C, the serological evaluation of CHV-1 circulation started after three cases of major neonatal mortality (Table 3).

Table 3. Reproductive pathology associated with immune status in kennel C

No. #	Breed	Gender	IFA result	No of infertile matings	No of litters	Litter size	Neonatal mortality
1.	Havanese Cuban Bichon	F	–	0	3	15	0
2.	Yorkshire Terrier	F	–	0	2	12	6
3.	Yorkshire Terrier	M	–	-	-	-	-
4.	Havanese Cuban Bichon	F	–	1	0	0	0
5.	Havanese Cuban Bichon	F	–	1	0	0	0
6.	Bichon Maltese	F	+	0	2	10	4
7.	Yorkshire Terrier	F	+	0	2	10	6
8.	Havanese Cuban Bichon	M	–	-	-	-	-
TOTAL				2			16

In the kennel C, they are eight breeding animals from three breeds (Bichon Maltese, Havanese Cuban Bichon, and Yorkshire Terrier). Despite the limited number of animals, the reproductive disorders were considerable, with 16 cases of neonatal mortality from the 47 new-borne puppies. However, serological evaluation for CHV-1 revealed the presence of specific antibodies only in two bitches with neonatal mortality (#6 and # 7). Neonatal mortality in bitch #6 (Bichon Maltese breed) was 40% and in bitch #7 (Yorkshire Terrier breed) was 60%. The intensity of signs was quite similar in both cases. However, the negative result obtained in bitch #2 cannot exclude the involvement of CHV-1 in neonatal mortality. Also, here cannot be excluded the CHV-1 infection in all the negative dogs of the contaminated kennel. Previous studies have shown that CHV-1 is a weak immunogenic virus and detectable antibodies can be recorded within 2-3 weeks after infection (Takumi et al. 1990). Even more, antibodies decrease quite rapidly and cannot be detected after few months (Carmichael and Greene 1998).

The reproductive disorders recorded in kennels A, B and C are graphically represented in figures 1, 2 and 3.

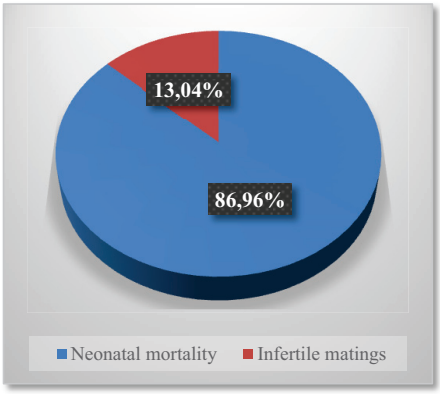

Figure 1. Reproductive disorders recorded in kennel A

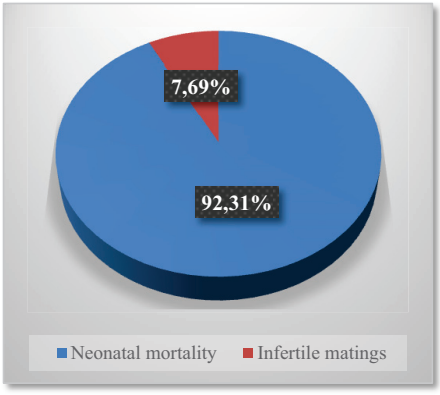

Figure 2. Reproductive disorders recorded in kennel B

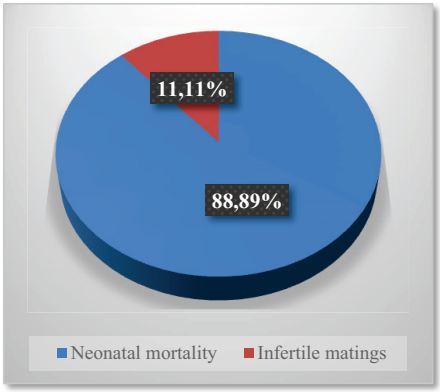

Figure 3. Reproductive disorders recorded in kennel C

In all kennels, neonatal mortality covered 90% (72/80) of reproductive disorders, and infertility 10% (8/80).

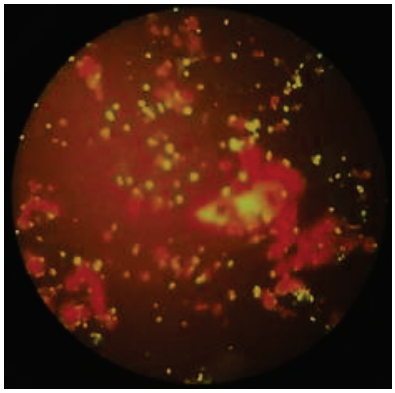

Figure 4. Positive IFA-CHV-IgG result in a female dog with a recent history of neonatal mortality (dilution 1:80; magnification 400X)

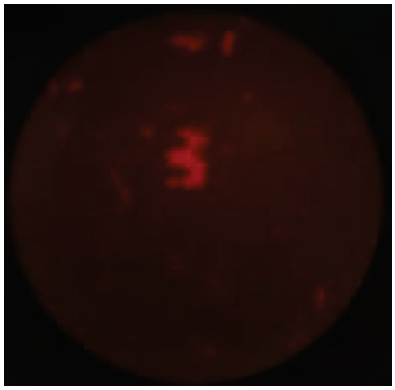

Figure 5. Negative IFA-CHV-IgG result in a female dog without a history of reproductive disorders (dilution 1:80; magnification 400X)

This study was the first approach in order to assess the presence of CHV-1 infection in dog breeding kennels from the Bucharest-Ilfov area. As resulting following the serologic assays and, as to be expected, in this area too, the dogs have been exposed to CHV-1. In Romania, similar studies have been done by Chielaru (2014) in five Northeast counties: Bacau, Galati, Iasi, Suceava, and Vaslui. Research on seroprevalence of canine herpesvirus-1 infection in northwest Romania revealed 20.55% (37/180) seropositive animals. The highest seroprevalences were in Iasi (27.77%) and Vaslui (26.86%) and lowest in Bacau (15.38%), Galati (11.11%) and Suceava (5.00%) (Chielaru, 2014). In our survey, the average of seropositive animals are being 86.36%, ranging from 100% in kennel A (Table 1) and B (Table 2) to 25% in kennel C (Table 3). Previous Romanian CHV-1 seroprevalence data are much lower than those obtained in this study, but it is not relevant to compare those data because the subjects investigated have different status. In the Chelaru (2014) survey were investigated several solitary lifestyle animals and in our study were investigated only breeding animals in kennels. Dog populations with 100% positive titres were reported in Finnish breeding kennels, facing reproductive problems, while lower values were obtained in breeding kennels without reproductive problems (65%) (Dahlbom et al., 2009). Our data are quite similar with other European serological surveys that shown high prevalence in households or breeding kennels in Belgium (45.80%) (Ronsse et al., 2002), England (88.00%) (Reading and Field, 1999), Italy (27.9%) (Sagazio et al., 1998), Lithuania (26.88%) (Musayeva et al., 2013), the Netherlands (39.30%) (Rijsewijk et al, 1999), and Turkish (62.1%) (Yesilbag et al., 2012).

In our survey, reproductive disorders were reported mainly in females that provided a positive result in the serological test for CHV-1. There were also serologically positive female CHV-1 who did not show reproductive disorders, as did serologically negative female CHV-1 who had reproductive disorders. These cases, even if singular, require the extension of investigations to other aetiologies of reproductive disorders but also to the protective effect on gestation of anti-CHV-1 antibodies.

CONCLUSION

Our study emphasizes the widespread of CHV-1 infection. This recommends the assessment of the immune status of the animals before their breeding season. The breeding kennels should benefit from a protocol of surveillance and prophylaxis for those infectious diseases impairing their health status and reproductive performance.

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SYRINGOSUBARACHNOID SHUNT PLACEMENT IN A CAVALIER KING CHARLES SPANIEL DOG DIAGNOSED WITH CHIARI LIKE MALFORMATION AND SYRINGOMYELIA. CASE REPORT

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Abstract

The aim of this paper was to describe the syringosubarachnoid shunting as surgical management in Chiari like malformation and Syringomyelia, and to track the outcome of the patient. A Cavalier King Charles Spaniel female dog was referred for neurological evaluation due to the intense sudden attacks of pain, occurring more and more often lately. A year earlier, the dog was diagnosed with Chiari-like malformation and syringomyelia, undergoing to a craniocervical decompression with durotomy. Postoperatively the clinical signs improved only for a short period of time, afterwards the clinical symptoms progressively reappeared, worsening in the last two months, thus requiring another surgical intervention. The dog was subjected to surgical placement of a syringosubarachnoid shunt at the cervical spinal cord, the patient being evaluated periodically during the hospitalization, and after that at one, four and twelve months postoperatively. In the follow-up period the patient presented good results with improvement of the clinical signs.
This case report highlights the success of cervical syringosubarachnoid shunt placement in the management of canine Chiari-like malformation with syringomyelia, especially when the other therapeutic options were unsuccessful.

Key words*: Chiari like malformation, dogs, syringomyelia, syringosubarachnoid shunt, magnetic resonance imaging.*

INTRODUCTION

The Cavalier King Charles Spaniel (CKCS) is a small, popular toy dog, originated in the United Kingdom. In Romania the breed presented a grown in popularity in the last ten years, CKCS dogs are sweet-tempered, good with children, playful, and affectionate.
Unfortunately, this breed presents a high predisposition to develop Chiari-like malformation and Syringomyelia.
Chiari like malformation (CM) and syringomyelia (SM) represents an important complex disorder of the nervous system, commonly encountered in the Cavalier King Charles Spaniel dogs, but it can also be diagnosed in other brachycephalic and toy breeds, such as Griffon Bruxellois, Affenpinschers, Yorkshire terriers, Maltese, Chihuahuas, Pomeranians, Boston terriers, Papillons and their crosses (Marino et al., 2012; Rusbridge, 2013).
Canine Chiari like malformation resembles the human Arnold-Chiari type I malformation, a congenital disorder in which the mismatch between the caudal fossa volume, which is too small, and its contents, lead to herniation of the cerebellum and sometimes also the brainstem into or through the foramen magnum (FM), resulting in cerebrospinal fluid flow disturbance (Freeman et al., 2014; Rusbridge, 2007; Rusbridge, 2014).
CM may lead to the development of fluid filled cavities (named also syrinx) within the parenchyma of the spinal cord, condition known as syringomyelia.
Most of the times Chiari-like malformation and syringomyelia evolve together, however, the conditions can evolve independently of the other, affecting dogs aged between 6 months to 10 years old (Rusbridge, 2014; Platt, 2004; Zachary et al., 2011).
The complete mechanism for the development of SM is not fully understood, the most popular theory is that the cavities are formed secondary to the disrupted cerebrospinal fluid (CSF) flow and inconstant CSF pressure (DeLahunta et al.,

2009; Rusbridge, 2014; Cerda-Gonzalez et al., 2009).

The common clinical sign encountered is scratching in the air near the neck - „phantom" scratching, which is the reason why the condition is known as "neck scratcher's disease". Other important sings are the neuropathic pain, expressed by yelping after a sudden posture change, frequent and characteristic manifesttation in CM/SM, along with the head, neck and spinal discomfort (Plessas et al., 2012; Platt, 2004; Rusbridge, 2014; Park et al., 2015)

A variety of non-specific signs accompanies the clinical presentation of CM/SM, including behavioural changes, hyperesthesia, cervical scoliosis, thoracic and pelvic limb ataxia, "bunny-hopping" pelvic limb gait, proprioceptive deficits, vestibular signs and facial nerve paralysis (Rusbridge, 2007; Plessas et al., 2012; Platt et al., 2012).

The gold standard to diagnose CM/SM is magnetic resonance imaging scan (MRI), so that accurate measurements of the caudal cranial cavity can be performed, including the position and shape of the cerebellum, the extension and the symmetry of syringomyelia can also be determined (Platt, 2004; Rusbridge, 2014).

British Veterinary Association (BVA) proposed a CM/SM classification scheme. Based on the position of the cerebellum, CM is structured in 3 grades, from grade 0 - no signs of CM, to grade 2, where the cerebellum is severe herniated through FM.

There is also a grading system for SM, according on the syrinx size there are 3 grades of SM, grade 0 SM – normal spinal cord, to grade 2 SM, where the syrinx diameter is greater than 2 mm (Freeman et al., 2014; Rusbridge, 2014).

The CM/SM grading is important, because it will guide treatment and prognosis.

MATERIALS AND METHODS

A 4-year-old Cavalier King Charles Spaniel female dog was referred for neurological evaluation due to the intense sudden attacks of pain, occurring more and more often lately. The patient was diagnosed a year before with CM and SM, and at that time a craniocervical decompression was performed. After the surgery the patient presented a favorable evolution in the first three months, with the improvement of the clinical signs. Unfortunately, after this period the clinical signs progressively deteriorated over time. In the last two months the dog presented a progressive neuropathic pain, expressed by sudden yelping, neck and spinal discomfort, scoliosis, apathy and depression. Besides these clinical signs, the patient also presented „phantom" scratching and "bunny-hopping" pelvic limb gait.

Due to the medical history and progressive clinical signs, we performed an MRI scan of the brain and spinal cord. Sagittal and transverse T1 and T2 weighted images were acquired. The scan revealed cerebellar herniation, with secondary FM obstruction – corresponding to grade 2 CM (Figure 1).

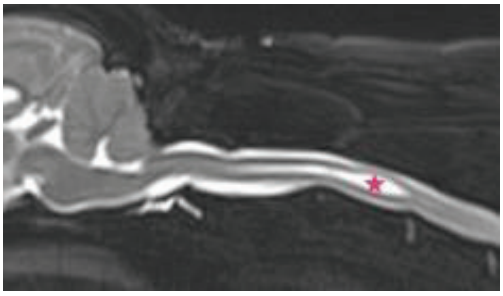

Figure 1 – T2 weighted mid-sagittal image of the caudal cranial fossa and upper cervical spinal cord. The cerebellar vermis is impacted into foramen magnum. Note the syrinx present in the cervical spine (red star).

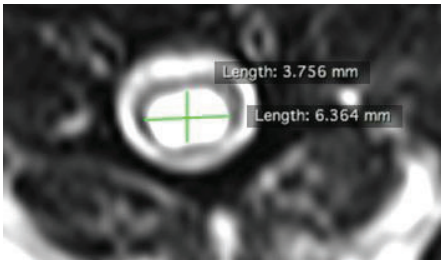

Figure 2 – T2 weighted transverse image of the cervical syrinx. The maximum width of the spinal fluid-filled cavity measures 6.3 mm.

The maximum syrinx width is located cervical, (Figure 1 and Figure 2), marking the position where the shunt will be surgically placed. The width of the syrinx is measured in a transverse plan, in this case the maximum internal diameter is larger than 2 mm, corresponding to grade 2 SM (Figure 2).

The dog was subjected to surgical placement of a syringo-subarachnoid shunt. Prior to the surgery, pre-medication of the dog was performed with 20 mg/kg intravenous cefazolin sodium (Iespor, I.E. Ulagay), 0.03 mg/kg intravenous medetomidine hydrochloride (Domitor, Orion Pharma, Pfizer Animal Health) and 0.04 mg/kg intravenous atropine sulfate (Atropin 0,2%, Vetaş). Anesthesia was induced with 4 mg/kg intravenous propofol (Propofol 1%, Fresenius Kabi), and maintained with isoflurane 1 to 2% (Forane, Baxter) in 100% oxygen. Fluid therapy (0,9% sodium chloride injection USP, Baxter) was administered during the entire surgical procedure in constant rate infusion at 10 ml/kg/hour. Postoperatively analgesia was provided with intravenous 4 mg/kg tramadol hydrochloride (Contramal 100 mg, AbdiIbrahim, Grunenthal) at every 12 hours, for 5 days. After the surgical intervention cefazolin was administered in dose of 20 mg/kg, for 7 days, and in order to decrease the neuropathic pain gabapentin was given for 2 weeks, in dose of 10 mg/kg PO orally, every 8 hours.

The dog was positioned in sternal recumbency on a special operating table, which allows to flex the patient's neck at 60 degrees. The cutaneous region of interest is aseptically prepared, the first step of the surgery is to perform dorsal laminectomy at C3-C4 (Figure 3), where the syrinx presents the largest width, previously measured on the magnetic resonance imagining examination.

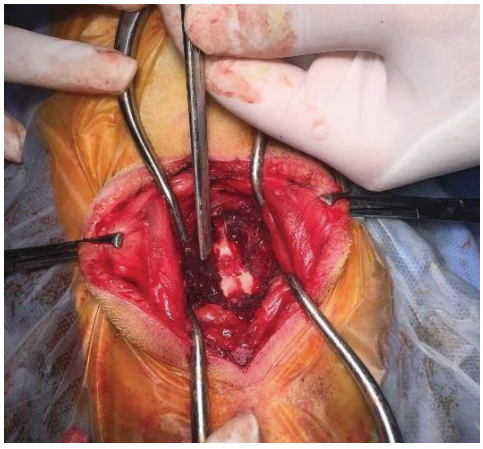

Figure 3 - Intraoperative aspect, performing dorsal cervical laminectomy.

A ventriculoperitoneal catheter was used to create the syringosubarachnoid shunt. Due to the syrinx size, the catheter is tailored to approximately 2,5 cm length (Figure 4), at both terminal ends of the catheter three fenestrations were made, in order to ensure adequately drainage of CSF. One loop is made on the middle of the tube, using nylon thread, having the role of anchoring the tube to the intervertebral ligament.

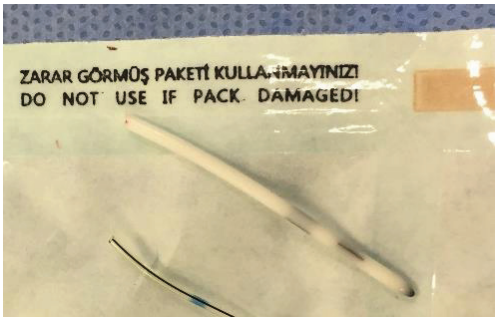

Figure 4 – The catheter used to create the syringosubarachnoid shunt is tailored from a ventriculo-peritonal catheter.

Dura mater and arachnoid are incised with an 11 scalpel blade, presenting an elongated triangular blade, which allows to obtain a thin and precise incision.

The spinal cord is incised in the dorsal midline, penetrating the syrinx. One end of the catheter was introduced into the lumen of the syrinx, in a cranial direction (Figure 5).

The shunt is secured using the nylon loop from the middle of the tube, suturing the shunt to the intervertebral ligament.

The protruding end of the catheter was caudally inserted into the subarachnoid space, thereby achieving the communication between the syrinx lumen and the subarachnoid cavity (Figure 6).

Dura mater was not sutured; homologous adipose tissue was placed over the laminectomy defect. Suture of the muscle, subcutaneous and skin layers was routinely carried out.

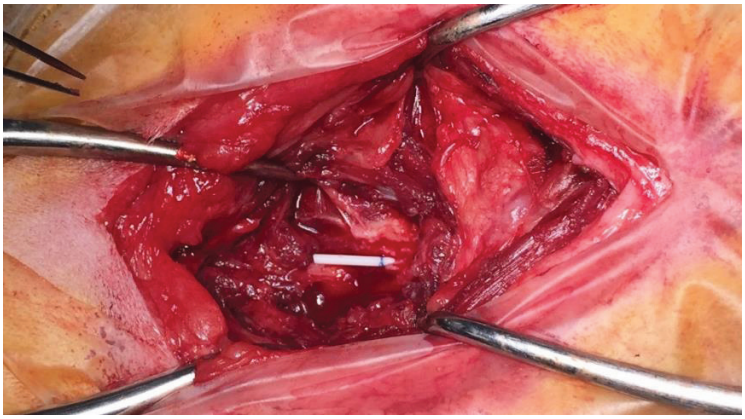

Figure 5 - Intraoperative aspect - one end of the catheter was cranially placed in the spinal cavity. Note the nylon loop present on the middle of the shunt.

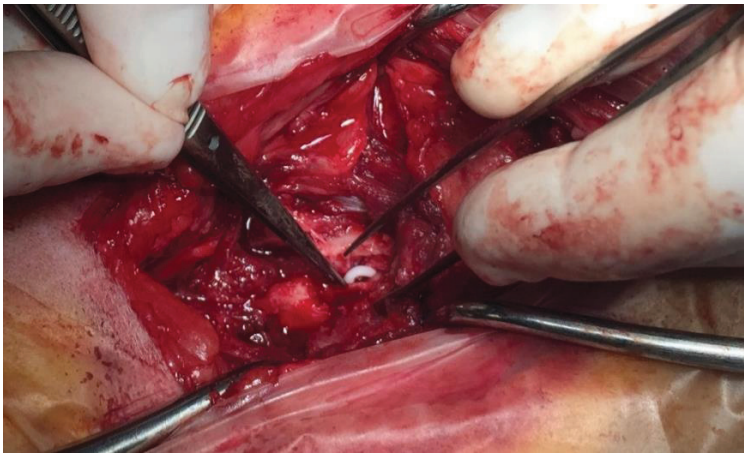

Figure 6 - Intraoperative aspect, orientation of the free-end catheter towards the subarachnoid space, achieving the syringosubarachnoid communication.

RESULTS AND DISCUSSIONS

The MRI scan revealed cerebellar herniation, a wide medullar syrinx located cervical, with the maximum width at the level of the third and fourth cervical vertebrae. In the transverse plan, the maximum internal diameter of the syrinx is 6,36 mm, corresponding to grade 2 SM. Postoperative MRI was not performed.

The syringosubarachnoid shunt was placed at the level of C3-C4, the surgery was successful, no intraoperative or postoperative complications were noted.

We choose to manufacture the tube that we used for shunting from a ventriculo-peritonal catheter, witch fitted optimum to the desired dimensions, and it is flexible and thin. The tube should allow the CSF passage and must be big enough to avoid debris occlusion. In a study performed by Motta in 2012, he chooses to use an equine ocular lavage tube, due to its size, cost and proprieties. The surgical technique that he used resembles, but he did not anchor the shunt to the intervertebral ligament (Motta et al., 2012).

In the early postoperative follow-up period the patient presented a mild neck pain, only for 24 hours. The dog did not presented ataxia, gait abnormalities or proprioceptive placement deficits. After 5 days the dog was discharged, and evaluated after that at 1, 4 and 12 months after the syringosubarachnoid shunt placement.

The neurological examinations in the follow-up period showed progressive improvement of the clinical signs, the neurological pain decreased, and the painful episodes are fewer and have low intensity.

At three years after the syringosubarachnoid shunt placement the patient presents an amelioration of the clinical signs comparative to the preoperative period, notably decreased neuropathic pain, the intensity and the frequency of the pain attacks reduced, scratching and yelping diminished, overall increasing the life quality of the patient.

The main purpose of the treatment is to reduce the pain. There are two treatment options for CM/SM, medical therapy or surgical intervention, which are elected according to clinical symptoms, and can be combined (Rusbridge, 2014).

The most common used surgical technique is craniocervical decompression, which can provide a satisfactory quality of life for a period of time between a few months and two years postoperatively, according to the CM/SM grade and also to the clinical signs. Another surgery technique described is syringosubarachnoid shunting procedure, with a better outcome for patients. Syringosubarachnoid shunt placement can be an option with good results, especially for those patients in which foramen magnum decompression has failed (Motta et al., 2012; Rusbridge, 2014).

Currently the scientific literature offers more information about craniocervical decompression technique, syringosubarachnoid shunting representing a relatively new procedure used in dogs. In patients that had craniocervical decompression, although the syrinx size still persists after this procedure, clinical amelioration is mostly due to the improvement of the CSF flow (Park et al., 2015; Rusbridge, 2014).

In human medicine better results were noticed in patients that underwent syringosubarachnoid shunt placement compared to the ones that underwent only foramen magna decompression or syringoperitoneal shunting. The syringosubarachnoid shunt placement has favorable results regarding the pain control, this technique is preferred to be used in patients with large syringes (Hida et al., 1995).

CONCLUSIONS

Chiari like malformation and syringomyelia represents a complex and progressive neurologic condition, in whose diagnosis and surgical management the magnetic resonance imagining plays an essential role.

Syringosubarachnoid shunt placement represents a safe and efficient procedure, which leads to clinical improvement of the clinical signs and reestablishes the quality of life.

Syringosubarachnoid shunting can be an alternative option with good results, especially in cases in which foramen magnum decompression has failed.

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MYASTHENIA GRAVIS IN A LABRADOR RETRIEVER DOG – CASE PRESENTATION

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Abstract

The patient, Labrador Retriever, male, eight years old, was presented at veterinary doctor showing muscle weakness, walked heavily on hind limbs and muscle contractions. The first signs of muscle weakness appeared five months ago, they have progressively worsened and 14 days before the consult it stopped barking. No changes were observed in biochemical and hematological blood tests as well as imagistic examinations, but the neurological examination revealed the decrease of spinal reflexes in all four limbs. Myasthenia gravis was suspected and the diagnosis was based on clinical signs and the favorable response to administration of neostigmine 1mg / kg intravenously.

Key words*: myasthenia gravis, muscle fatigue, neostigmine*

INTRODUCTION

Myasthenia gravis is an autoimmune, neuromuscular disorder and it is manifested by an attack against acetylcholine receptors from the post-synaptic membrane of the neuro-motor plaque, causing their degradation or blockage, resulting in motor deficiency and skeletal muscle weakness. (Burns, 2010, Hotineanu and Stasiuc 2010, Marx et al., 2015, Thomas, 2014).

It may have genetic determinism or can be acquired (Bexfield et al.,2006, Dissanayake et al.,2016, Herder et al.,2017).

Myasthenia gravis is characterized by a decrease in the number of acetylcholine receptors (AchR) from the postsynaptic muscular membrane and by flattening or simplifying postsynaptic fold crests. These defects lead to a diminished efficacy of neuromuscular transmission, and even if normal AchR release occurs, the post-synaptic potentials produced are of low power and do not trigger the potentials of muscle fiber action, resulting in insufficient transmission of information in a significant number of neuromuscular junctions, which leads to the weakness of muscle contraction. Neuromuscular abnormalities of myasthenia gravis are induced by an autoimmune response mediated by specific anti-AchR antibodies. These antibodies reduce the number of AchR available in muscle junctions through three different mechanisms: AchR can be degraded more rapidly through a mechanism involving their cross-coupling followed by accelerated endocytosis; the active sites of AchR, where they normally bind to Ach, may be blocked by antibodies, the postsynaptic muscle membrane may be damaged by complement-associated antibodies (Bexfield et al., 2006, Ciobanu, 2011).

Symptomatology of myasthenia gravis is clinically expressed by abnormal weakness of volunteer muscles at resting restraining with restoration to rest, ocular signs (palpitations), dysphagia with nasal regurgitation of liquids, fallout of the mandible, weakening of the neck and neck muscles, weakening of the pelvis, difficulty walking (Dissanayake et al. 2016, Fraser et al., 1970, Shelton, 2016). However, muscle weakness is not always associated with physical effort. Muscular weakness at the facial, pharyngeal or esophageal level is common, and in many situations there is a mega-esophagus in the absence of generalized muscular weakness (focal myasthenia), so megaesophagus may be a primary affection or a symptom (Thomas, 2014).

Paraclinic investigations are an indispensable adjunct to the clinical examination, in many cases having diagnostic certainty (Fraser et al., 1970).

Myasthenia gravis occurs with a higher frequency in adult dogs, especially in the

German Shepherd, Golden Retriever and Labrador Retriever, Jack Russell Terrier (Blakey et al., 2017) and is rare in cats (Shelton, 2016).
Diagnosis is often delayed, due to the rare frequency of the disorder and due to manifestations that may be confused with various metabolic or neurological pathologies (Ciobanu, 2011).
Antibodies to acetylcholine receptors can be detected in the serum of sick animals by indirect immunohistopathological examination using normal muscle as a substrate (Marx et al., 2015, Thomas 2014), the diagnosis of certainty is established after detection of antibodies in the serum, but about 2% of cases with generalized myasthenia gravis may be seronegative (Shelton, 2002).
The most common complications are regurgitation and aspiration pneumonia (Thomas 2014).
Generalized muscle weakness disappears rapidly after i.v. of edrophonium chloride (0.1-0.2 mg / kg), and this treatment is often used as a diagnostic test. Administration of short-acting anticolinesterase (edrophonium chloride) produces a dramatic increase in muscle strength (Thomas 2014).
Treatment consists of administering long-acting anticolinesterase and immunosuppressive for the chronic condition. The treatment is anticholesterase drugs - pyridostigmine (1-3 mg / kg, PO, bid-tid) or neostigmine (0.04 mg / kg, SC, qid). Immunosuppressive doses of prednisolone and other immunity-modulatory drugs are recommended in animals that do not respond to anti-hemostasis and / or chronic disease. Prognosis is usually good, and in many cases remission is spontaneous, and is proven by lowering the antibody titer. Prognosis is reserved for animals with persistent muscle weakness or aspiration pneumonia, which is the leading cause of death or euthanasia (Atiba et al., 2014, Thomas 2014).

MATERIALS AND METHODS

In November 2017, a Labrador, 8, year-old, chocolate colored dog, was presented at the FMVB Medical Clinic for showing changes in the rear train and no improvement in anti-inflammatory treatments for the past 4-5 months. The patient lives in and out of the house and has a 9-year-old Labrador female partner. It is not castrated and was vaccinated until it was 5 years of age, internally and externally deworming. The clinical examination was performed and it was revealed that the voice was altered, the owner stating that it was not barking, trying, but it sounds very raucous. It tired quickly.
The neurological examination revealed:

- status: present,
- consumption: cheerful
- posture: prefers decubitus in the sterno-abdominal
- cranial nerves: fallen inferior eyelids, "menace" reaction delayed in both eyes
- panniculus delayed on both sides
- normal perianal reflex
- normal spinal flashes, but the hind limbs trembled
- proprioception - in the "robber" and "flexion-extension" examinations, the reactions were delayed and difficult.

When examining walking:

- kyphosis,
- walks a few steps then sits,
- intolerance to effort. It takes a few steps and then sits down. Walking gets harder and harder, the rear train's muscles get tense and the dog starts jumping, then it sits on the hind limbs, then lateral or abdominal decubitus.

Diagnosis: suspicion of myasthenia gravis based on exercise intolerance and fatigue while walking.

RESULTS AND DISCUSSIONS

With symptomatic, supportive and immune-suppressive treatment in case of adverse response to symptomatic treatment, prognosis is favorable (Gilhus and Verschuuren, 2015).
The therapeutic trial with myosin (neostigmine bromide) was performed intravenously. 3 minutes after administration, the patient walked normal.
The therapeutic trial is used in human medicine and commonly used in veterinary medicine to diagnose myasthenia gravis (Gilhus and Verschuuren, 2015). The patient progressed favorably and therapy was instituted with Mestinon cp 60 mg, 1 mg / kg at 6 h p.o.

Supportive treatment has been recommended for mild physical activity and a diet to prevent weight gain. At the following check-up, it has been found that it was walking normal, but it also had slightly dropped eyelids. Continued treatment with Mestinon 60 mg, 1 mg / kg at 8 hours and moderate physical activity. After 3 months it returns to control and investigation.

CONCLUSIONS

Radiological imaging should be performed as soon as possible after the occurrence of clinical signs to exclude megahexophagus. Symptomatic treatment with neostigmine bromide will be instituted immediately, for the patients to have a good quality of life. Doses are based on the body's response to treatment, paying attention to the side effects of the drug. The patient should be monitored periodically. The diagnostic and treatment algorithm should include the in-situ strict examination of patients with myasthenia gravis in order to institute effective treatment.

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HEMATOLOGICAL CHANGES ASSOCIATED WITH SUBCLINICAL MASTITIS IN GOATS

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Abstract

The aim of this study was to investigate haematological changes in lactating goats with subclinical mastitis. Determination of mammary health status was based on CMT results, microbiological and cytological examination. Blood samples were taken from all goats included in the study by venopuncture of the jugular vein and blood was collected in vacuum blood collection tubes. The results showed that the mean ± SE in healthy animals and goats with subclinical mastitis were as follows: Red Blood Cell count (RBC) 10.43 ± 0.63×10^{12}/L and 9.38 ± 0.42×10^{12}/L; Haemoglobin (Hb) 85.69 ± 2.43 g/L and 77.13 ± 1.73 g/L; Hematocrit (HCT) 18.77 ± 0.87 % and 17.77 ± 0.68 %; Red Blood Cell Distribution (RDW) 21.55 ± 0.16 % and 22.21 ± 0.13 %; Mean Cell Volume (MCV) 16.96 ± 0.29 fL and 15.63 ± 0.20 fL; Mean Cell Haemoglobin (MCH) 6.18 ± 0.10 pg and 6.73 ± 0.09 pg; Mean Cell Haemoglobin Concentration (MCHC) 379.81 ± 3.72 g/L and 378 ± 3.12 g/L; White Blood Cell Count (WBC) 13.37 ± 1.60×10^{9}/L and 16.66 ± 1.23×10^{9}/L. The RBC and Hb were significantly higher (P<0.05) in normal lactating compared to goats with subclinical mastitis. RDW, MCH and WBC count was significantly lower in healthy goats.

Key words*: Hematological parameters, goats, subclinical mastitis.*

INTRODUCTION

Inflammation of the mammary gland in lactating productive animals is one of the main diseases causing significant economic losses for farmers. Mastitis in small ruminants in its subclinical form is estimated with an annual prevalence of 5-30% (Bergonier et al., 2003). Diagnosis is based on the use of various indicators of inflammation and isolation of microorganisms, most commonly coagulase-negative staphylococci being isolated (Wilson et al., 1995). The major changes include the migration of ions, proteins and enzymes from the blood into the milk due to an increased blood vessel permeability and active phagocytic invasion occurrence, resulting in an increase in cellular composition and reduction of several components in the milk. All of the affected halves produce specific inflammatory substances - acute phase proteins, which can also be used as indicators of an inflammatory process.
Somatic cell levels and the isolation of microbial agents is accepted as the "gold standard" in the diagnosis of subclinical mastitis. In goats, the level of somatic cells as an indicator for mammary gland infection should be carefully interpreted. Overall, goat milk contains a higher amount of cytoplasmic particles and epithelial cells than cows (Paape and Capuco, 1997), due to the peculiarities of milk secretion in this species. The existence of a correlation between somatic cell level and California mastitis test (CMT), has been investigated by other authors (Persson and Olofsson, 2011). According to Contreras et al. (1996) scores 0 and 1 show 79% of uninfected halves, and in the 2-3 score there is an increase in somatic cells and suspicion of infection, which requires sampling for microbiological examination.

MATERIALS AND METHODS

Animals

This study used 32 locally bred goats, between 3 and 7 years of age, from licensed farms in Bulgaria. Milking was done manually, herds were free of brucellosis, tuberculosis and mycoplasmosis. All of the animals were clinically healthy and free from internal and external parasites. Their health status was evaluated based on rectal temperature, heart rate,

respiratory profile, appetite, faecal consistency. The animals were divided into two experimental groups. In group A (healthy animals) included only animals with both mammary halves with CMT score (-) or (+). Group B (with subclinical mastitis) included only animals in which both mammary halves had a CMT score (++) or (+++). In additional microbiological and cytological study of these samples, the diagnosis of subclinical mastitis was confirmed.

Sample collection

Blood samples were collected from all animals, by venopuncture of the jugular vein with closed blood collection system in vacuum tubes, containing EDTA. Samples were transported in ice box to the laboratory for analysis. Haematological analysis included red blood cell count (RBC), haemoglobin (Hb), hematocrit (HCT), red blood cell distribution (RDW), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), white blood cell (WBC), count using BC-2800 haematology analyzer (Mindray, China).

All udder halves of the lactating animals were examined using a CMT-Test (Kruuse, Denmark) to detect subclinical mastitis. The CMT reagent reacts with DNA of epithelial and inflammatory cells present in the milk. CMT results were read immediately and were scored for each teat depending on the amount and thickness of gel formed. In this study, CMT scores of '0' and 'trace' were considered as negative or normal while CMT scores of 1 (weak positive), 2 (distinct positive) and 3 (strong positive) were taken as indicators of subclinical mastitis.

Statistical analysis

Statistical analysis was performed using SPSS 16.0

RESULTS AND DISCUSSIONS

Table 1 shows the results of the CMT test of the animals included in the study. According to these results, the first group (healthy), included 9 negative (-) and 7 weak positive (+) goats. The second group (with subclinical mastitis) included 12 animals with (++) and 4 with (+++). Diagnosis of subclinical mastitis was confirmed in the previous microbiological and cytological study of these samples (Hristov et al., 2016).

Table 1. Results of CMT analysis of goats

	Investigated	CMT score			
		(-)	(+)	(++)	(+++)
goats	32	9	7	12	4
halves	64	19	13	26	6

Table 2. Results of haematological analysis of goat blood samples

Parameter		*healthy goats*	*with subclinical mastitis*
RBC x 10^{12}/L	*Mean ± SE*	10.43 ± 0.63*	9.38 ± 0.42
	Min	5.41	6.63
	Max	14.84	12.91
Hb g/L	*Mean ± SE*	85.69 ± 2.43*	77.13 ± 1.73
	Min	65	68
	Max	98	89
HCT %	*Mean ± SE*	18.77 ± 0.87	17.77 ± 0.68
	Min	11.5	11.9
	Max	22.6	20.4
RDW %	*Mean ± SE*	21.55 ± 0.16	22.21±0.13*
	Min	20	21.4
	Max	22.4	23.3
MCV fL	*Mean ± SE*	16.96 ± 0.29	15.63 ± 0.20
	Min	15.5	14.3
	Max	18.8	16.8
MCH pg	*Mean ± SE*	6.18 ± 0.10	6.73 ± 0.09*
	Min	5.5	6.1
	Max	6.6	7.3
MCHC g/L	*Mean ± SE*	379.81 ± 3.72	378 ± 3.12
	Min	359	360
	Max	402	396
WBC x 10^9/L	*Mean ± SE*	13.37 ± 1.60	16.66±1.23*
	Min	7.8	8.2
	Max	18.9	23.5

* $P \leq 0.05$ statistically significant difference between groups

Table 2 illustrates the haematological parameters for healthy and goats with subclinical mastitis. The ranges and means ± SE of haema-

tological parameters were as follows: RBC x 10^{12}/L (A) 5.41 – 14.84 and 10.43 ± 0.63; (B) 6.63 – 12.91 and 9.38 ± 0.42; Hb g/L (A) 65 - 98 and 85.69 ± 2.43; (B) 68 - 89 and 77.13 ± 1.73; HCT % (A) 11.5 – 22.6 and 18.77 ± 0.87; (B) 11.9 – 20.4 and 17.77 ± 0.68; RDW % (A) 20 – 22.4 and 21.55 ± 0.16; (B) 21.4 – 23.3 and 22.21 ± 0.13; MCV fL (A) 15.5 – 18.8 and 16.96 ± 0.29; (B) 14.3 – 16.8 and 15.63 ± 0.20; MCH pg (A) 5.5 – 6.6 and 6.18 ± 0.10 (B) 6.1 – 7.3 and 6.73 ± 0.09; MCHC g/L (A) 359 - 402 and 379.81 ± 3.72 (B) 360 - 396 and 378 ± 3.12; WBC x 10^9/L (A) 7.8 – 18.9 and 13.37 ± 1.60; (B) 8.2 – 23.5 and 16.66 ± 1.23.

The mean RBC and Hb averaged in this study were statistically higher in healthy animals than those with subclinical mastitis. At the same time, MCH, RDW and WBC values were statistically higher in goats with subclinical mastitis. The MCV, HCT and MCHC values did not show statistically significant differrences.

Although there is a significant decrease ($P \leq 0.05$) in RBC and Hb in animals with subclinical mastitis, those parameters are within the normal limit for this species (Douglas J. Weiss and K. Jane Wardrop, 2010). This indicates that the mentioned blood indicators are not substantially affected by the inflammation. The broad range of measured values as well as the difference in the two groups of animals can be explained by the influence of many additional factors such as nutrition, habitat, environment, age, reproductive status and stress. (Zumbo et al., 2011; Waziri et al., 2010). Changes in the mean values of MCV, MCH, MCHC and RDW, relative to the reference values for healthy goats, are insignificant and have no clinical relevance. This is confirmed by the results of other studies (Piccione et al., 2014), reporting values in healthy goats similar to ours or even higher.

A significant increase ($P \leq 0.05$) of WBC was observed in goats affected by subclinical mastitis compared to healthy animals. A similar increase in WBC with an increase in absolute number of monocytes, eosinophils and neutrophils has also been reported in lactating cows in other studies (Alhussien et al., 2015; Sarvesha et al., 2016). WBCs increase as a result of the invasion and spread of pathogenic microorganisms in the mammary gland and systemic reaction of the body. At the same time, such increase in WBC is not always found in all lactating animals (Dang et al., 2007; Khaled et al., 2015). This is indicative that in some cases the process can be localized only in the mammary gland without systemic response. A possible explanation for these findings could be the different immunological status of the animals and the degree of pathogenicity of microorganisms causing subclinical mastitis.

CONCLUSIONS

The haematological parameters in goats with subclinical mastitis are mainly expressed by the elevation of the WBC levels. Changes in other parameters (RBC, Hb, MCV, MCH, MCHC, RDW) are insignificant in relation to healthy animals and have no clinical relevance.

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MICROSCOPICAL AND PHYSICO-CHEMICAL ASPECTS OF THE COMPOSITION AND INTEGRITY OF RAW DRIED SALAMI WITH NOBLE MOULD

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Abstract

Raw dried salamis with noble mould are among Romania's most popular meat food products. The assumption that there might be an unknown side to the structural integrity of the ingredients in such products has developed over time. A physico-chemical analysis was carried out on six salami samples of various brands of the raw dried salamis known as "Salam de Sibiu" and "Salam de Hateg", both available on the retail market. The following parameters were measured: humidity percentage, nitrites content, NaCl content, easily hydrolysable nitrogen, percentage of fat and amount of protein. All samples underwent the histopathologic examination routine procedure with paraffin and HE (haematoxylin and eosin) stain. The values of the integrity parameters comply with the applicable legislation, but vary significantly from a producer to another. The histopathologic examination indicated the presence of animal tissue and plant structures. We consider this examination to be useful in identifying structural non-compliance.

Key words*:* raw dried salamis, physico-chemical analysis, histopathologic examination.

INTRODUCTION

Salami is dried fermented sausage consisting of pork mixed in different proportions with beef, mutton or horse meat with different additives, represented by salt, spices and curing salt (Feiner, 2006).

Salami was produced for the first time in Italy more than 270 years ago (Leistner, 1986).

The European countries recognized for the production of salami are: Italy, Germany, Spain, France and Hungary. They produce several hundred million kilograms of salami per year (Bertolini et al., 2006).

The suitable technology is selected according to climatic conditions, as seen in the Mediterranean region and Southern Europe, where meat products are dried to lower the amount of water taking advantage of long dry and sunny days, while in Northern Europe fermented sausages require smoke for subsequent preservation (Toldra, 2014).

In Northern and Central Europe, smoked salami is preferred, and maturation is controlled by the addition of acid-starter cultures, while in Southern Europe, salami is slowly dried in the air and it is often mould-maturated. Preparations that are typical for a region or area have specific characteristics derived from the use of ingredients and local production techniques that are deeply rooted in tradition and linked to the territory where they come from (Aquilati et al., 2016).

In Romania, among the most popular varieties of raw dried salami we mention: Banat salami, Bacau salami, Sibiu salami and Hateg sausages (Mencinicopschi et al., 2006).

The Sibiu salami is a type of salami that is made of raw meat. Due to the artificial climate conditions this product can be manufactured in any part of the country throughout the year. The materials used for the Sibiu salami are pork and pork fat.

The pig has to be healthy, to have a reduced degree of contamination and to be properly refrigerated. It must neither be too young, nor too fat. It needs to have a certain ratio of water/protein and fat/protein content, to be rich in heminic compounds (myoglobin), to have a low amount of connective tissue and to have an optimal water retention capacity (the PSE-pale, soft, exsudative and DFD- dark, firm, dry meat

is excluded). Also, boars, pregnant sows or animals fed with oleaginous plants or fish flour are rejected. In the production of the Sibiu salami are used only half-carcasses from pigs that weigh over 130 kg, belong to the Mangalita breed and are degreased and refrigerated for minimum 72 hours before processing. The fat used must have increased consistency and high freshness. The mixture of spices can be made according to different recipes or according to the requirements of the beneficiary It can include the following: sodium chloride, glucono-lactone, carbohydrates, ascorbic acid/ ascorbates, organic food acids (citric, lactic, tartaric), enibahar, garlic, sugar, and starter cultures (Comanaru, 2000).

The quality of the Sibiu salami depends directly on the quality of raw material as it is raw dried salami that is processed only by cold smoking and maturing by drying, without the use of thermal treatments.

The histological method and physico-chemical analysis are the mostly applied techniques for detecting unauthorized tissues in meat products. Sezer et al. (2013) showed in their study that in a type of sausage they found animal hair of root of hair, spleen, esophagus and epithelium of sensory organs, tissues that should not exist in this food product (Sezer et al., 2013). In another study also using a histological approach Malakauskienė et al. (2016) discovered nerve tissue, fat and blood vessels in canned sausages (Malakauskienė et al., 2016).

Therefore, the physico-chemical and histological evaluation of the composition of this type of meat preparation is essential for the quality assessment.

The aim of the present paper is to evaluate the quality of the Sibiu Salami by analysing the regular physico-chemical parameters and by performing the histological analysis of the composition in order to emphasise the particularities derived from each method in terms of product integrity and composition assessment.

MATERIALS AND METHODS

Physico-chemical analysis were carried out on six salami samples of various brands of raw dried salami known as “Salam de Sibiu” and “Salam de Hateg”, both available in the retail chain. The analysed samples of “Salam de Sibiu” are similar in terms of formulation. However, “Salam de Hateg” distinguishes by the inclusion of beef mixed with pork and a hydrolysed vegetable protein (Table 1).

Table 1. Label composition of analyzed samples

Sibiu salami	Sample A	Sample B	Sample C
label composition	pork, fat, salt, sugars, spices, ascorbic acid, sodium nitrite	pork, fat, salt, sugars, spices, ascorbic acid, brandy 0.4%, sodium nitrite	unknown recipe
Sibiu salami	Sample D	Sample E	Sample F
label composition	pork, fat, salt, sugars, spices, ascorbic acid, sodium nitrite, starter culture	pork, fat, salt, dextrose, spices, sodium ascorbate, sodium nitrite	pork, fat, salt, dextrose, spices, ascorbic acid, sodium ascorbate, sodium nitrite, starter culture
Hateg salami	Sample G		
label composition	beef, pork, soybean protein, salt, sugar, spices and spice extracts, corn hydrolysed protein, sodium ascorbate, carmine, sodium nitrite, starter culture		

The following analyses were performed: moisture content, nitrites content, collagen/ protein ratio, NaCl content, easily hydrolysable nitrogen, lipids and proteins content.

From each sample 200-300 g were taken according to protocol, from the middle and the ends of the bars (Purcărea, 2015).

The samples were previously prepared according to the methods that were going to be used and with the results that were going to be obtained.

The determination of moisture content was obtained by oven drying.

The determination of the nitrite content was performed by Griess method according to the SR EN 12014-3:2005.

For the determination of total nitrogen and total content of protein the Kjeldahl method was used, according to SR ISO 937: 2007.

The content of easily hydrolysable nitrogen was indirectly determined by titration with NaOH according to SR 9065-7: 2007.

The content of lipids was determined by using the Soxhlet method.

The regulations for the minimum and maximum values of the parameters pursued in this study for the product groups mentioned above are found in The Order MARD 560 / 16.08.2006 and the means of conducting the analyses are described in ISO Reference Standards. In order to obtain high accuracy results, the analyses were performed at a laboratory accredited by RENAR.

Table 2. Physico-chemical parameters of analyzed samples

No.	Sample	Moisture (%)			nitrites (mg/kg)			NaCl (%)			Easily hydrolyzable nitrogen (mg NH_3/100g)			Fats (%)			Proteins g/100g		
		Reg*	Result	Literature	Reg.**	Result	Literature	Reg*	Result	Literature	Reg.*	Result	Literature	Reg.*	Result	Literature	Reg*	Result	Literature
1.	A	30	29.17	39,8 [1]	150	0.79	1-6 [7]	6	4.10	4.32 [1]	200	73.61	63.59-176.3 [8]	46	38.86	18.5-31.1 [9]	20	23.79	31,3 [1]
2.	B	30	25.66	36,25 [2]	150	0.54	0.6-3.4 [9]	6	4.04	4.70 [2]	200	129.46	17.3-32.03 [9]	46	40.31		20	25.78	25,76 [2]
3.	C	30	27.59	48,6 [3]	150	0.38		6	3.95	3.8 [3]	200	64.99		46	39.83		20	24.21	15,5 [3]
4.	D	30	27.47	30,5 [5]	150	0.43		6	5.26	5.50	200	129.48		46	40.35		20	25.80	20,3 [5]
5.	E	30	29.51	75,45 [6]	150	0.56		6	3.80	4.90	200	89.78		46	38.81		20	29.19	21,29 [6]
6.	F	30	26.4		150	0.53		6	5.35	1.72-1.96 [9]	200	95.97		46	39		20	25.83	
7.	G	35	31.89	38,00 [4]	150	0.45		6	3.9	4.32 [4]	200	43.36		50	38.83		16	23.44	29,7 [4]
	Average		28.24			0.53			4.34			89.52			39.43			25,43	
	Std. dev.		2.11			0.1337			0.6647			32.1954			0.7112			1.9448	

(*Ord. 560/2006; ** Ord. 438/295/2002; [1]Zanardi et al., 2010; [2]Casiraghi et al., 1996; [3]Van Schalkwyk et al., 2011; [4] Demeyer, 2007; [5]Ockerman and Basu, 2007; [6] Conte et al., 2012; [7] Păduraru et al., 2010; [8] Jude et al., 2011; [9]Dobrinas et al., 2013)

The freshness of the samples, evaluated by the determination of easy hydrolyzable nitrogen was appropriate, the maximum limit of 200 mg NH3 / 100 g of product was not exceeded.
The values obtained ranged between 43.36-129.48 mgNH3 / 100 g, with a mean of 89.52 ± 32.1554.
As the standard deviation indicates, easy hydrolysable nitrogen values vary significantly from one sample to another. Nevertheless, a similar variation in the values was reported by other authors for similar products; Jude et al. (2011) communicating for the analysed samples an interval between 63.59 and 176.3 mgNH3 / 100g.
A significantly lower value for easily hydrolysable nitrogen was reported by Dobrinas et al., (2013) (the value of f is 21.93159, p is 0.000668, the results are statistically significant at $p < 0.05$) for pork and sheep samples, their study range being 17.3-32.03 mgNH3/100g, with an average of 26.76 ± 5.71 mgNH3/100 g.
The protein percentage was superior to the minimum value for the considered meat products category, the values for the studied samples ranging between 23.44-29.19%, with a mean of 25.43 ± 1.9448.
In literature, there are recorded much lower protein levels, such as 15.5% (Van Schalkwyk et al., 2011), but also higher, up to 29.7% (Demeyer, 2007), or even 31.3 (Zanardi et al., 2010).
Histological findings were revealed in various tissues: stranded muscle tissue (Figure 1), different types of conjunctive tissue, dominant fat tissue (Figure 2), vascular structures and nerve threads (Figure 3).

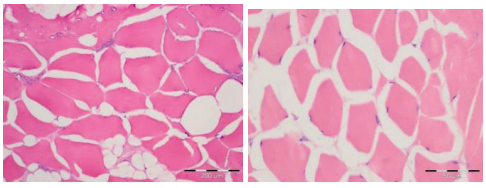

Figure 1. Left - cross-sectional muscle fibers and reduced area of adipose tissue (ob.10x, HE stain); Right - cross-sectional muscle tissue, homogenized muscle fibers and inconsistent spacing from the endomisium (ob.40x, HE stain).

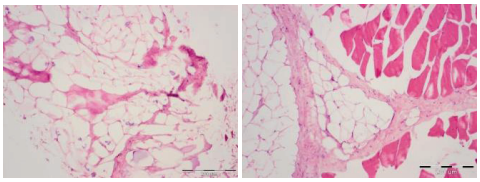

Figure 2. Left - Adipose tissue (ob. 10x, HE stain); Right - Adipose tissue, conjunctival stroma and obliquely sliced muscle tissue (ob. 10x, HE stain)

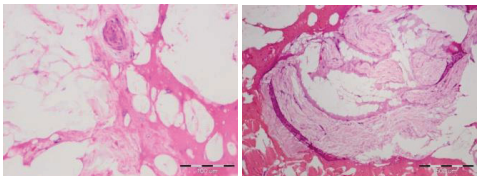

Figure 3. Left - Amorphous structure, adipose tissue and cross-sectional vessel (ob. 20, HE stain); Right - Muscle fibers and nerve threads (ob. 4x, HE stain)

In some sections there were found vegetal structures with different morphology and tincture than that of animal tissues (Figure 4).

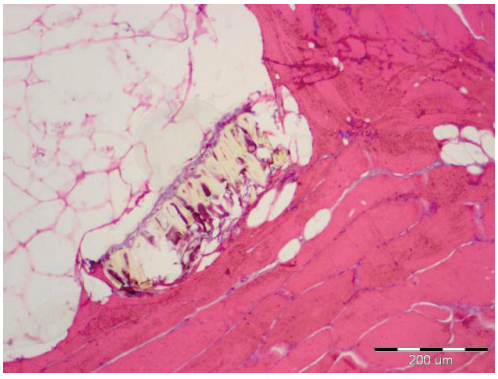

Figure 4. Muscle tissue and adipose tissue; fragment of plant fiber (ob. 10x, HE stain)

The composition and techniques of preparing a food product are key elements for its quality. Although the analyses show differences in physico-chemical properties from one sample to another, it is important that when notifying any changes to the original recipe, these should be redressed in order to preserve the quality of the product. The values of the integrity parameters comply with the applicable legislation, but vary significantly from one producer to another. These significant variations in parameter values integrity lead to important quality differences between products

that all fall into what is considered to be the premium category.

According to the histopathological examination, it is found that the products under examination show specific tissues of pork and pork fat. Vegetable structures are met either in the form of fibers or in the form of basophilic anchovy deposits. What has kept our attention is the homogeneous appearance of the muscular fibers and their inconsistent spacing from the endomisium. Appearance could be associated with the dehydration process following the treatment of meat with salt. The homogenization of the fibers without revealing contractile protein-specific striations may be an aspect associated with muscle tissue maturation and we consider that the integrity of the analyzed products is not negatively influenced.

CONCLUSIONS

The values of physico-chemical parameters are in accordance with the applicable legislation. However, statistically, they vary significantly from the same preparation analysed by other specialists.The histopathological examination indicated the presence of animal tissue and plant structures. The morphological analysis complements the data on the integrity and quality of raw-dried salami.

In accordance with the data recorded by literature, the present study does not find structures foreign to the salami recipe.

We consider this examination to be useful in identifying structural non-compliances.

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OPEN REDUCTION METHODS OF LUXATIONS IN DOGS AND CATS: A COMPARATIVE STUDY

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Abstract

BACKGROUND: The most frequent luxations encountered in small animal pathology are located at the humero-radio-ulnar joint and tibio-tarsal-metatarsal joint, usually with traumatic etiology.
OBJECTIVE: The aim of this study is to determine which elbow luxation reduction method has the best advantages, taking into consideration some specific variables such as long term prognosis, post-operative lameness and the extent of soft-tissue trauma caused by the open reduction techniques.
MATERIALS AND METHODS: Two surgical techniques have been used comparatively: a technique using screws and cerclage wire and one using modified bone staples. This study was conducted on 12 cases, of which six being treated using screws and cerclage wire and six using metallic implants.
CONCLUSIONS: Surgical reduction of traumatic luxations in dogs and cats using modified bone staples has a better outcome with minimal damage on joint dynamics and soft-tissue trauma and should be considered as a reduction method of choice for all breeds of dogs and cats, having lower surgical costs, a shorter interventional time and a better post-operative recovery.

Key words: *cat, dog, elbow luxations, orthopaedics, veterinary surgery.*

INTRODUCTION

Because of the bony anatomy of the region and the inherent stability provided by the muscular and ligamentous structures of the joint, traumatic luxation of the elbow joint is usually uncommon in dogs and rare in cats (Tobias and Johnston, 2012).
Strong collateral ligaments (CL) provide stability and the anconeal process interlocking within the olecranon fossa prevents luxation when the joint is extended (Schaeffer, 1999).
The ulna is prevented to move medially by the medial epicondyle of the humerus, but the rounded shape of the lateral epicondyle permits the anconeal process of the radius to move off the lateral epicondylar crest when the elbow is flexed more than 90 degrees Medial luxations are usually accompanied by severe ligamentous damage (Piermattei et al., 1997). Recognition of collateral ligament damage is important for optimal management of elbow luxation. Diagnosis of rupture is based on physical examination after closed joint reduction and the need for further operative stabilization based on those findings (Billings et al., 1992). Integrity of the collateral ligaments could be assessed in the dog by the range of pronation and supination achieved during manual rotation of the manus with the elbow and carpus both flexed to 90 degrees to position the anconeus caudal to the olecranon fossa so that rotary joint stability was provided primarily by the CLs (Campbell, 1971).
Rupture or avulsion of the medial collateral ligament (MCL) increases pronation to 100 degrees, whereas rupture of the lateral collateral ligament (LCL) increases supination to 120-140 degrees (Farrell et al., 2007).
Closed reduction of luxations should first be attempted in elbow joints with good stability, but when this is not possible and ligaments are ruptured or avulsed, open reduction and stabilisation should be considered.
Most of recent literature has focused only on canine elbow luxation with just one report of a traumatic elbow luxation in a cat. This study has the purpose of evaluating presentation, post-operative lameness, the extent of soft-tissue trauma and limb function in two separate open reduction techniques for both dogs and cats with traumatic elbow luxation.

MATERIALS AND METHODS

The study was conducted on 12 cases, 9 dogs and 3 cats, with traumatic elbow luxation caused by car accidents, high-rise syndrome and dog attacks, treated by using two different open reduction techniques. Records of all animals were reviewed for history, clinical examination findings and treatments provided.

Diagnosis

Diagnosis of traumatic elbow luxation was made using both clinical and radiological findings. Patients had a history of recent injuries, such as car accidents or high-rise syndrome. All patients were non-weight bearing on the affected limb and carried the forelimb in a characteristic position - the antebrachium in abduction and external rotation and the elbow joint in slight flexion. The elbow was swollen and painful when palpated, revealing a proeminent radial head, an indistinct lateral humeral condyle and a lateral displacement of the olecranon. The range of motion was limited. Diagnosis is confirmed on a craniocaudal (CrCd) radiographic projection where a lateral displacement of the radius and ulna is apparent. The lateral view shows an uneven joint space between the humeral condyle and the radius and ulna. Considering the traumatic cause, patients were assessed for concurrent injuries before surgery.

Surgical management

As soon as preoperative assessments were complete and no contraindications were identified, the patients were anesthetized safely for both closed and open reduction procedure. If previous closed reduction failed to reduce elbow luxation, open reduction procedures were applied according to clinical status of the animal.

The surgical techniques used in this study consisted in placement of screws at the attachment site of the collateral ligament in both humeral epicondyle and radial neck and a cerclage wire looped around each screw in a figure of eight pattern or placement of modified bone staples in the radial head and the humeral epicondyle.

After surgical reduction and stabilization, the joint is evaluated for stability and radiographs are taken in order to assess bone alignment and implant positioning. A soft padded bandage is applied for 24 to 48 hours to improve the patient's comfort and to minimize soft tissue swelling.

Aftercare

In patients with mild to moderate post-reduction instability, the standard of care recommended consisted in providing external support in the form of a (spica) splint or a soft-padded bandage for at least 5 to 7 days. After removal of bandage or splint, owners were encouraged to initiate rehabilitation measures.

Six weeks post-operatively, owners were contacted by telephone and were asked to answer questions regarding function of the affected limb, 'excellent' being used to describe animals with no noticeable lameness, 'good' for slight lameness and 'poor' for failure to use the leg or in case of serious to severe lameness. (Table 1). Owner satisfaction was rated as ‘satisfied’ or ‘not satisfied'.

Table 1. Clinical parameters used for assessment of post-operative results

	Excellent	Good	Poor
Lameness	No lameness	Slight lameness	Serious to severe lameness
Weight-bearing	Full weight bearing	Weight bearing to tolerance	Standing with no weight bearing
Limitation of joint movement	No limitation of joint movement	Mild limitation of joint movement	Moderate to serious limitation of joint movement
Pain on movement	No pain on motion of affected joint	None to slight pain on motion of affected joint	Moderate to severe pain on motion of affected joint

RESULTS AND DISCUSSIONS

Individual case results for 9 dogs and 3 cats in this study are presented in Table 2.

Epidemiology and clinical signs

The study included several small and medium dog breeds, with a mean weight of 15.3kg, mostly being males (66%).

Table 2. Summary of signalment, clinical findings and treatment of the 12 cases presented with elbow luxation

Case no.	Breed	Sex	Age (years)	Weight (kg)	Concurrent injuries	Open reduction type	Stability following reduction	Lameness score
Dogs								
1	Bichon maltese	M	4.0	4.0	Radius and ulna fracture	Screws and cerclage wire	Good	Good
2	Crossbreed	M	2.0	25.0	None	Modified bone staple	Stable	Good
3	Crossbreed	F	7.0	22.0	Superficial wounds	Modified bone staple	Good	Excellent
4	Yorkshire terrier	M	0.1	1.0	None	Screws and cerclage wire	Good	Excellent
5	Beagle	M	4.0	18.0	None	Screws and cerclage wire	Stable	Poor
6	Crossbreed	F	6.0	15.0	None	Modified bone staple	Good	Excellent
7	Crossbreed	F	3.5	21.5	None	Screws and cerclage wire	Stable	Good
8	German shorthaired pointer	M	4.5	20.7	Superficial wounds	Modified bone staple	Good	Excellent
9	Crossbreed	M	11.0	10.5	Cranial dislocation of radius	Screws and cerclage wire	Stable	Poor
Cats								
1	DSH	F	2.0	3.0	None	Modified bone staple	Good	Excellent
2	DSH	M	0.3	1.5	Superficial wounds	Modified bone staple	Good	Excellent
3	Norwegian Forest cat	M	5.0	4.2	None	Screws and cerclage wire	Good	Good

Two of the cats in the study were Domestic Shorthairs (DSH), with a mean weight of 2.25 kg and one was a Norwegian Forest cat, two females and one male.
Most of the luxations were caused by car accidents (7 cases, 58%), other causes included high-rise syndrome and dog attacks. As concurrent injuries we found 3 cases of superficial wounds, one radius and ulna fracture and one cranial dislocation of radius. All cases sustained lateral elbow luxation, with obvious collateral ligament damage on physical examination (Figures 1 and 2).

Treatment

General anaesthesia was applied for closed reduction in order to achieve the profound muscle relaxation needed for manipulating the elbow into position. Neuromuscular blockage may be helpful in cases of concurrent fractures.
Closed reduction was the method of choice for all animals. If closed reduction failed to reduce elbow luxation, open reduction techniques were used.
Open reduction is also indicated in cases of avulsion, fractures of the origins of the collateral ligaments, articular fractures, intra-articular interposition of soft tissues, chronic luxations, marked instability or reluxation after closed reduction.
Anatomic landmarks for open reduction of elbow luxation are the radial head, olecranon and anconeal processes and lateral humeral condyle (Fossum, 2013).
The patients are positioned in lateral recumbency and the leg is prepped from the shoulder to the carpus.
Half of the patients were treated using a surgical technique that consists in exposing the elbow by using a limited approach to the head of the radius and lateral compartments of the elbow joint. This method is best suited in luxations with ligament damage but it can also be used in cases of minimal ligament damage.
Following reduction, stability can be enhanced by primary repair of the collateral ligament. If the ligament has torn from its attachment to the bone or it is completely destroyed it can be replaced using two screws, one in the humeral epicondyle and one in the radial head and a figure eight cerclage wire looped around each of them (Figure 3).
The other half of the patients was treated using a surgical technique that consists in a lateral, smaller approach of the elbow joint.
After reducing the luxation, a modified bone staple is used, one part of the implant being shorter than the other. The shorter part is inserted in the radial head and the longer one in

Figure 1. Lateral luxation of elbow in a 5yo Norwegian Forest cat, CrCd view

Figure 2. Lateral elbow luxation in a 3.5yo Crossbreed dog, lateral view

Figure 3. Reduced elbow luxation in a 4yo Beagle using the screws and cerclage wire surgical technique, CrCd view

the humeral condyles (Figures 4, 5 and 6). This method is used when there is minimal ligament damage, in order to increase stability of the joint and prevent relaxation.

Aftercare

The elbow is most stable when moderately extended to the normal standing angle of 140 degrees (Piermattei et al., 1997). After reduction, in order to maintain stability, a soft-padded bandage with lateral splint can be used for about 5 days. Exercise should be restricted within the house or leash for about 2 weeks post-operatively. Exercises of flexion and extension should be started after removal of the bandage in order to regain mobility of the joint. In cases of ligament damage, a more rigid immobilization is needed, using a spica splint, maintained for about 2 weeks. Exercise is restricted within the house or leash for 3-4 weeks. After splint removal, exercises of flexion and extension of the joint need to be started (Mitchell, 2011).

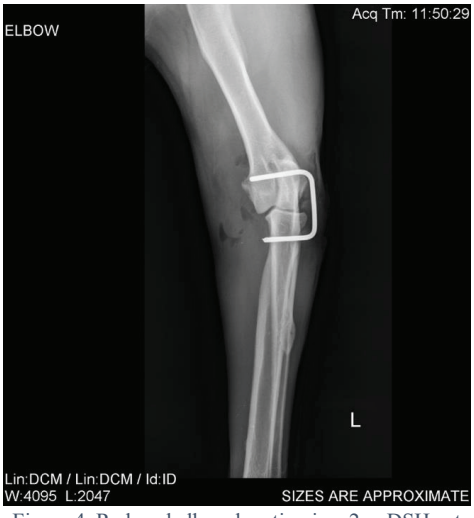

Figure 4. Reduced elbow luxation in a 2yo DSH cat using bone staples, CrCd view

Figure 5. Reduced elbow luxation in a 6yo Crossbreed dog using a modified bone staple, CrCd view

Figure 6. Reduced elbow luxation in a 6yo Crossbreed dog using modified bone staples, lateral view

Post-reduction results

After 6 to 9 weeks, all owners were contacted by telephone and were asked to rate the outcome of the intervention. 4 dogs and 2 cats were rated as 'excellent' (50%), meaning they regained full mobility after surgery, 3 dogs and one cat were rated as 'good' (33%), meaning they still had some slight lameness at the time we called and 2 dogs were rated as 'poor' (17%), meaning they had serious lameness of the affected limb.

Owners were mostly satisfied with the final outcome, except the owners of the 2 dogs that still hadn't regained full mobility.

CONCLUSIONS

Lateral luxation was diagnosed in all patients of this study, which is consistent with other studies that have reported between 92% and 100% (Billings et al., 1992; Schaeffer et al., 1999).

The aforementioned open reduction techniques had an overall good postoperative outcome (83% were either 'good' or 'excellent').

The method using bone staples is considered to be better than the one using screws and cerclage wire, with a better stability following reduction, a need for a shorter time of exercise restriction and a better post-operative lameness score and owner satisfaction. This should be considered the method of choice for open reduction of traumatic elbow luxations if there is minimal ligament damage.

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MRI FINDINGS OF THE CERVICAL SPINE IN 3 BEAGLE DOGS

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Abstract

Degenerative disc disease is a condition of the spine caused by dehydration and degeneration of intervertebral discs being an inevitable process with increasing age of the animals. These changes can lead to clinical and pathological diseases characterized differently, such as Hansen type II (protrusion) and Hansen type I (extrusion) disc disease.
Medical records of three Beagle dogs, aged 3-8 years, who were presented in the clinic of the Faculty of Veterinary Medicine of Bucharest with neurological features, were reviewed. Animals were evaluated by radiographic and MRI imaging. Radiographic examination was conducted in lateral incidence at the level of cervical segment. MRI images were obtained with a low field magnetic resonance of 0.3 Tesla, through: T1 SE (spin echo), T2 FSE (fast spin echo) and T1 sequences after the administration of contrast agent, dogs were positioned in lateral decubitus under inhalatory anesthesia.Radiographic examination revealed narrowing of the intervertebral space in two cases, at the level of C2-C3. MRI examination revealed intervertebral disc degeneration and dehydration at a total number of eight discs in the cervical segment from all three cases. Hansen type II (protrusion) compression on the spinal cord has been registered in one case at C3-C4 level, and in two cases was detected Hansen type I (extrusion) at C2-C3 level, with the movement of the disc fragments in the spine, confirmed by radiographic examination.
Magnetic resonance imaging has provided a useful, safe, non-invasive evaluation of the cervical spinal cord.

Key words*: dog, extrusion, MRI, protrusion.*

INTRODUCTION

Intervertebral disc herniation is a spinal cord disease commonly found in dogs, with two types of degenerate intervertebral discs hernias, namely intervertebral disc extrusion or Hansen type I and intervertebral disc protrusions or Hansen type II (Hansen, 1952; Bergknut et al., 2013). Both types of disc disease are associated with different pathological and clinical features (Decker et al., 2016). Extrusion of the intervertebral disc is characterized by a sudden and unexpected herniation of the degenerated and/or calcified nucleus pulp by a rupture of the fibrous ring, followed by the migration of the disc material into the vertebral canal. The usual affection is found in young dogs belonging to the chondrodystrophic breeds and is accompanied by an acute onset of clinical signs, which may range from cervical hyperesthesia to paraplegia (Hansen, 1952, Smolders et al., 2013). Protrusion of the intervertebral disc is characterized by the slow, progressive and focal extension of the fibrous ring and the longitudinal dorsal ligament into the vertebral canal. This type of disorder is common in older non-chondrodystrophic dogs, where clinical signs, such as paresis and limb ataxia, slowly settle (Hansen, 1952; Smolders et al., 2013).
In practice, it is very important to make an exact distinguish between the extrusion and protrusion of the disc before the treatment. Magnetic resonance imaging (MRI) is considered to be the best way to diagnose intervertebral disc disease in dogs because it is safe, non-invasive and offers high-resolution digital images in multiple planes. It also provides a good view of the parenchyma of the spinal cord and other vertebral structures (Hansen, 1952; Murthy et al., 2014).
The objectives of this study were to describe and establish the type of disc degenerative disease in 3 Beagle dogs evaluated by neurological, radiographic and magnetic resonance examination.

MATERIALS AND METHODS

The medical records of **3 dogs** from the Beagle breed (case 1: 5 years old, female; case 2: 3

years old, male; case 3: 8 years, male) were reviewed at the clinic of the Faculty of Veterinary Medicine from Bucharest with neurological signs. The clinical evaluation was done according to the methodology described in the specialized textbooks (Vlăgioiu and Tudor, 2012). Radiographic images were obtained from latero-lateral incidence with an Optimus Bucky Diagnost (Philips, Germany) using a focal length of 100 cm. The MRI examination was done using a VET MR GRADE with a power of 0.3 Tesla (ESAOTE, Italy) using a permanent magnet and dedicated coils. The protocols used to obtain the images consist of T1 Spin Eco (SE) and T2 Fast Spin Eco (FSE) in three planes (sagittal, transversal and dorsal) with a slices thickness of 3 mm and postcontrast images obtained in T1 sequence after administration of the intravenous contrast substance (Multihance, 0.2 ml/kg). For the MRI examination, the animals were positioned in lateral/sterno-abdominal decubitus and were anaesthetized using premedication protocol composed of one of the following: Diazepam, Butorphanol or Domitor, then induction with propofol (Propovet, IV), then intubated and maintained under general anesthesia with Isoflurane in 100% oxygen and adjusted in accordance with the depth of anesthesia, to obtain superior image quality and without motion artifacts.

RESULTS AND DISCUSSIONS

Clinical examination by general examination methods revealed pain in the cervical segment, and after the neurological examination a disc degeneration at the C2-T2 level was suspected, but other pathologies such as myelopathy, vascular disease or a trauma at that level have not been excluded. Neurological signs were represented by: limb ataxia and low proprioception of the anterior limbs.

The radiographic examination revealed the narrowing of the C2-C3 intervertebral space in cases 1 and 3 (Figure 1), suspecting a disc herniation, and in case 2 there were no radiographic changes.

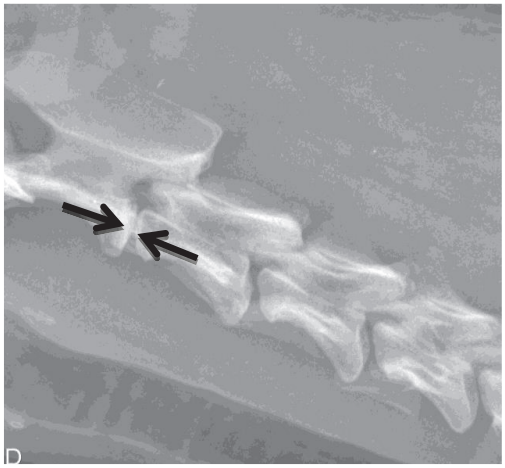

Figure 1 – Latero-lateral incidence of the cervical spine in case 3: collapse of intervertebral space at the level of C2-C3 (arrows) is observed

The magnetic resonance imaging examination revealed the following aspects: degeneration and dehydration of the intervertebral disc at a total of 8 discs in the cervical segment in all the three cases (case 1: 4 discs, case 2: 1 disc, case 3: 3 discs), from which one case (case 2) had disc protrusion (Hansen type II) at the C3-C4 intervertebral space with compression on the spinal cord (Figure 2), and in cases 1 and 3 disc extrusion was revealed (Hansen type I) at the C2-C3 level, as follows: median, right paramedian (case 1) with the caudal migration of the disc fragment in the vertebral canal (Figure 4 and 5), respectively (case 3) left paramedian disc extrusion (Figure 3). The results of the MRI examination confirm the radiography suspicion of disc herniation at the C2-C3 level.

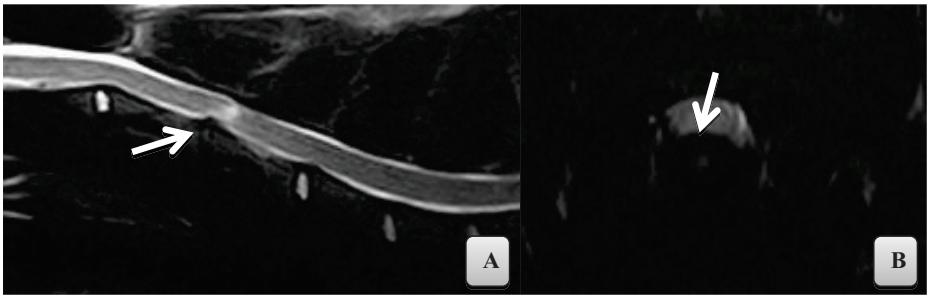

Figure 2 – Images in sagittal T2 (A) and transverse T2 (B) planes, at the C3-C4 intervertebral space, case 2: can be observed the hipointenity of the degenerate disc, dehydrated, but also moderate dorsal compression (A) respectively median compression of the meninges and spinal cord without breaking the fibrous ring (B). Light diffuse hiperintensity area caused by compression (arrows).

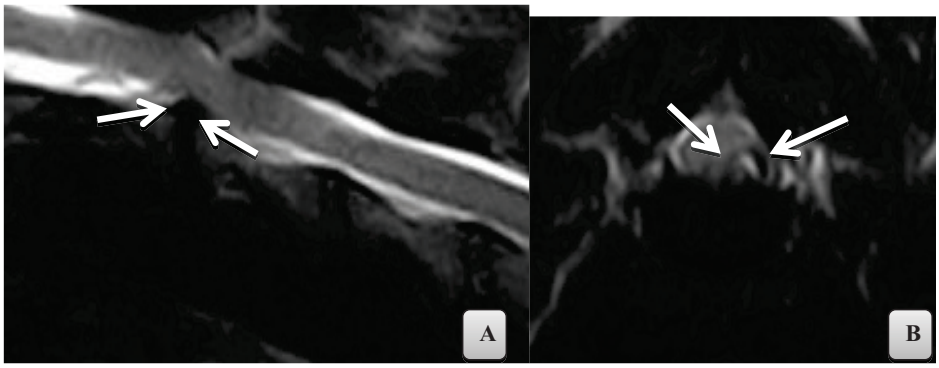

Figure 3 - Images in sagittal T2 (A) and transverse T2 (B) planes, at the C2-C3 intervertebral space, case 3: can be observed the hipointensity of the degenerate, dehydrated disc (A), but also a heterogeneous area, well-defined iso/hipointensity, left paramedian (B) compressing the spinal cord (arrows).

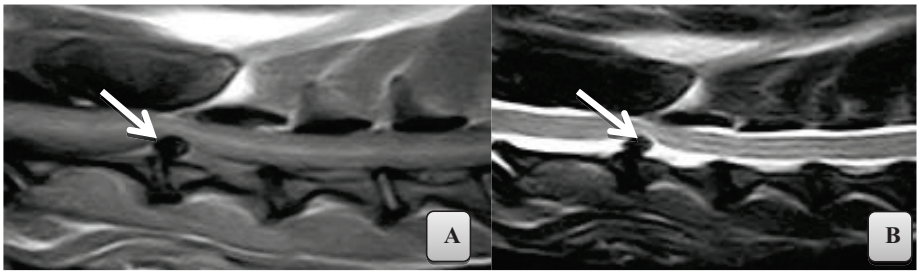

Figure 4 - Images in sagittal T1 (A) and T2 (B) planes, at the C2-C3 intervertebral space, case 1: can be observed the hipointensity of the degenerate, dehydrated disc, but also a heterogeneous area, well-defined hipointensity (disc material), slightly migrated caudally, compressing the spinal cord (arrows).

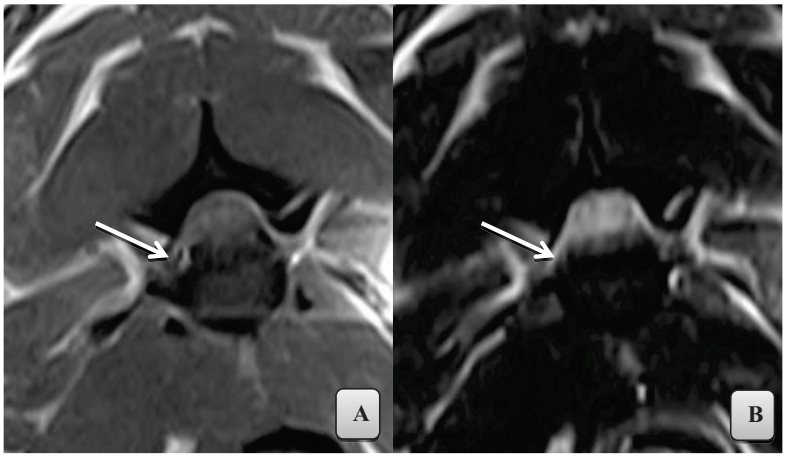

Figure 5 - Images in transverse T1 (A) and T2 (B) planes, at the C2-C3 intervertebral space, case 1: can be observed a slightly heterogeneous area, median, right paramedian, which disrupts the medullar parenchyma causing a medullary compression (arrows).

In the literature (Hansen, 1951, 1952), it is assumed that the disc degenerations in the condrodistrofic (CD) and non-condrodistrofic (NCD) breeds appears different depending on the age and the speed at which the degenerative changes spread, considering there are two different degenerative processes.

Further studies have supported this hypothesis, showing that there are important differences between the disc degeneration processes recorded in the CD breeds compared to those encountered in NCD breeds (Bergknut, 2013). In this paper are presented the imaging, radiographic and MRI aspects of three chondrodistrofic (Beagle) dogs (3-8 years old).

Intervertebral disc disease is a common cause of back pain and neurological deficits in dogs and in individuals witch present disc calcifications that increases the risk of intervertebral disc disease (Lappalainen A., and Col. 2001).

The clinical manifestations expressed by the animals that we evaluated were in agreement with previous data stating that intervertebral disc herniation caused different degrees of myelopathy, including pain, paralysis, or permanent paralysis, and the Beagle breed was most affected by the cervical intervertebral disc herniation (34.8%) (Itoh H., et al., 2008). It has also been shown that disc extrusion commonly affects small dogs in the cervical segment at C2-C3 and large at C6-C7 (Cherrone et al. 2004) or even C5-C6 and C6-C7 (Ryan and Col. 2008, Hillman et al., 2009, Costa Costa et al., 2012). In our report, disc changes have been identified at C2-C3 (case 1 and 3) and C3-C4 level (case 2), in concordance with previous studies.

In a study by Kranenburg et al. (2013), type I hernia has been reported to frequently affect the cervical segment (17/49), followed by the thoracolumbar segment (31/49) and the lumbosacral segment (1/49), while type II hernia frequently affects the lumbosacral segment (16/25), followed by thoracolumbar segment (5/25) and cervical segment (4/25), concluding that the cervical segment presents an increased risk of developing a type I disc disease and a low risk for a type II disc disease (Kranenburg et al., 2013).

Risk of occurrence and the development of intervertebral disc degeneration and their associated disorders is higher in chondrodystrophic breeds, suggesting that besides the potential genetic component, some external factors such as physical activity and the environment in which the animals live (Lappalainen A., and Column 2001).

CONCLUSIONS

In the presence of cervical pain, a neurological examination accompanied by a detailed imaging examination is needed to identify the presence of spinal cord injuries. Because cervical pain can have multiple causes, including intervertebral disc disease, magnetic

resonance imaging (MRI) is a useful method in confirming structural and positional alterations of intervertebral discs, thus excluding other pathologies and helping to guide the physician in choosing the appropriate therapy.

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A REVIEW OF ROBOTIC SURGERY EVOLUTION, CURRENT APPLICATIONS AND FUTURE PROSPECTS

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Abstract

The aim of this paper is to review the background of robotic surgery, the existing technology associated with this field, the main robotic surgical procedures, the training methods, the financial aspect of using these systems and future prospects for robotic-assisted surgery. Given the fact that robotic surgery has a history of only 30 years, the surgical possibilities are not researched to their full extent. The most appreciated feature of the robotic system is considered to be the high mobility and wide array of movement allowing access to do surgery on tissues inaccessible to classical approaches. With regards to the downside of this technique, it's almost unanimous accepted that it's cost related. To write this article over 60 literature sources about the history and development of surgical robots also concerning case studies, animal and human trials and their results obtained throughout the past few years were reviewed. Numerous trials that analyze new surgical applications are undergoing and although the system has been mainly used for soft tissue small interventions, its limits are yet to be determined.

Keywords*: animal model, da Vinci system, minimally invasive, robotic surgery.*

INTRODUCTION

Robotic surgery represents the newest and the most controversial type of minimally invasive surgery.

Although the first notions of robotic systems were mentioned for the first time centuries ago, robots were introduced to most fields like manufacturing, space exploration, research, transportation, in the past couple of decades, being just a matter of time for them to be designed for surgical purposes.

The development of new technology, at such a fast pace, brings new attributes to robotic systems and combined with the training of more and more surgeons in these techniques, it permanently modifies the existing data on advantages, disadvantages and possible uses of robotic surgery.

This review intends to offer complex and detailed data on the current situation of robotic surgery, analyzing multiple literature sources. Information about the background and beginning of robotic surgery, about the robotic systems available on the market, the training methods and about the procedures performed with this technology will be presented in this paper.

To write this article over 60 literature sources were reviewed. The papers were selected using specific criteria, using the following guiding points:

- Articles have to be published between 2000 and 2018;
- Articles have to be published as full papers;
- Particular keywords were used for each chapter of the review;
- Articles should have at least 15 references;
- Articles have to be written in English;
- Articles have to published from different medical settings;
- Their content had to be relevant to the subjects approached in this review.

All articles published before 2000, that only had an abstract presentation, that had less than 15 references, with no English translation and that had an irrelevant content were eliminated from reviewing. Over 200 articles were analyzed but only 62 met the criteria.

Robotic surgery is becoming more and more popular amongst the minimally invasive surgical centers, numerous hospitals already using this system or being in the process of implementing such protocols.

Due to its rising medical popularity both amongst doctors and patients, a paper that gathers the most recent data on this subject was thought necessary. Being a new field that develops at such a fast pace, the need for meta-analysis is high, idea supported by the fact that one of the most mentioned drawbacks of adopting this system is the poor literature on this subject associated with the lack of feedback.

History and evolution of robotic surgery

The use of robotics in surgery is first mentioned in 1985, which makes the history of this field only 33 years long. "Robota" is a Czech word used to describe forced labor and it was used to define artificial people almost a century ago. Although terms like "robotics" and "robots" are first used associated with science fiction literature and movies, these words were taken over by the scientific departments of several research fields, robots being designed for multiple purposes. Surgical robots have an impressive history, with multiple events and historical premieres during a short amount of time (Fig. 1). Da Vinci envisions a humanoid model over 500 years ago, for which he uses several mechanical details. His work on this human-shaped mechanical model inspires the Intuitive Surgical company to name their product the "da Vinci robot", which is currently high-end surgical robotic technology.

Imagined at first to be used in out of reach areas such as outer space, deep underwater or to be used around dangerous materials and substances, the robots were reinvented once the microelectronics and digital imagining have developed at an increasing pace, making robotics suitable for the surgical field. The da Vinci robot is the most used surgical robot, due to its multiple functions and characteristics (Lanfranco et al., 2004).

The telesurgical system was intended for different purposes, in the beginning, gradually being adapted to the popular system that it is today. This technology drew attention to it from the start. In 1972 NASA was considering telepresence surgery as a solution to provide surgical support to astronauts.

At that point in time, the technical development couldn't allow this project to be implemented. The telesurgery concept was further developed and made tangible the remote manipulation of scopes and different types of instruments. The Defense Advanced Research Projects Administration (DARPA) also shown interest in this technology, as was seen as a viable option to perform surgery on soldiers in areas that surgeons can't have immediate access. Several years later, this system was developed for commercial use, bringing it into minimally invasive centers around the world.

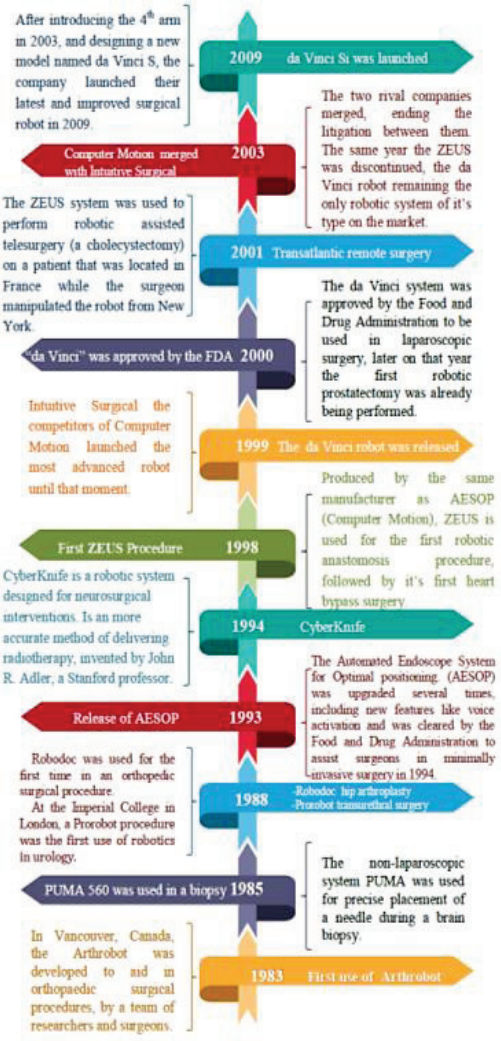

Figure. 1. The timeline of surgical robots development

The Intuitive Surgical Corporation launches the da Vinci robot in 1999 as groundbreaking medical technology, although the ZEUS robot, that was built on a similar concept, was already in use but with fewer characteristics (Shah et al., 2014).

The main robotic systems

Robotic surgery can be divided into 3 types, considering the surgeon's implication during the procedure:

- supervisory controlled: robotic arms execute the procedure following a predetermined program;
- telesurgical: remote surgery is conducted by the robotic arms manipulated from a distance by a specialized surgeon;
- shared-control: the surgeon performs the procedure using robotic arms as an aid, due to their characteristics of accurately manipulating the instruments.

Most medical robots were designed for a certain purpose, to be used in a certain type of procedure or for a specific medical field like neurosurgery, urology, gynecology, but every device is envisioned to extend the human skills, to compensate for surgeon errors, but not to replace the doctor's role in medical procedures.
Another way to classify the robotic systems is by their procedural role:

- passive role;
- restricted role;
- active role.

The current tendency is to use robots with active role that move more of the responsibility on the machines, decreasing this way the load that was supported by the surgeons.
The main robotic systems used until present day are the following:

- *Cyberknife* is an autonomous device that accurately positions and delivers radiation therapy to a tumor, guided by preloaded CT images correlated with real-time x-ray images.
- *Aesop* is a robotic system with voice control, that manipulates an endoscope. It has a restricted role because it's used only for visual support.
- *Robodoc* is a robot used in orthopedic procedures, specifically designed for hip replacement. This system is used for a certain part of this complex procedure, its role being bone drilling.
- *Acrobot* is another example of the use of robotics in orthopedic surgery. It has a similar role to Robodoc but it's used in the total knee arthroplasty procedure.
- *NeuroMate* is one of the first medical robots used in surgery, being also a system guided by preoperative images, that has as the main characteristic the ability to precisely position instruments, eliminating the human hand error.
- *ZEUS* is a robot used in laparoscopic and thoracoscopic surgical procedures. It has three robotic arms, one of them being strictly used for the endoscope, being an AESOP arm with voice control, while the other two are used to manipulate surgical laparoscopic instruments. The voice commands refer to the position of the endoscope, asking it to move vertically or horizontally until the command "stop" is given. This system had added a flexible wrist device. The telesurgical technology used here was improved and replaced by the one utilized in the da Vinci robot.
- *Da Vinci robot* is the most recent and complex robotic system and with its recent updates has become the most desirable technology for more and more minimally invasive surgical centers (Camarillo et al., 2004).

Given the wide array of possible procedures, correlated with all its features, often when robotic surgery is the subject of a debate, the use of the da Vinci robot is implied. This is why explaining how medical robots work will be made on this particular one.
Unlike all its predecessors, da Vinci it's the first that was approved by the FDA to be used in general laparoscopic surgery.
It is a teleoperated system, which implies that the surgeon manipulates instruments and scopes attached to the robotic arms, from a console placed at a distance from the actual surgical field (Freeman and Towle, 2015).
This technology is not intended to be autonomous, but to act as an extension of human capabilities, the surgeon being the one to control every movement of the instruments. Because of this particularity, these systems are often called "master-slave manipulators".

During the brief history of this technology, only ZEUS and da Vinci systems were approved to assist in surgery and nowadays only the latter is still produced, making it the sole robotic device of its type (Morris, 2005).
The da Vinci system has three main components:

- the surgeon's console;
- the patient-side cart;
- the visual tower.

The surgeon's console (Fig. 2) is the interface between the surgeon and the robot and has multiple functions such as:

- The console viewer offers HD images of the surgical site, with the possibility of magnification up to ten times;
- The master controllers are also called fingertip controllers, being manipulated by the surgeon's hands. The motion scaling feature is one of the most appreciated characteristics allowing the adjustment of hand-to-instrument movement ratios. Another great feature associated with the master manipulators is the hand tremor filter which removes any unwanted movement (Bodner et al., 2004);
- The footswitch panel is used to perform other tasks such as switching between instruments or adjusting the light;
- Numerous ergonomic settings are designed to offer the surgeon comfort during surgery;
- After the latest upgrades, the newer da Vinci robot offers the dual console feature, a technical innovation that allows collaboration between surgeons, being also a great training asset.

A widely cited disadvantage of robotic systems is the absence of tactile feedback (Wottawa et al., 2015).
The patient side cart represents the main robotic component with four active arms. Endowrist instruments can be attached to the robotic arms that control them (Fig. 3). Changing the instruments require the help of a medical professional. A wide selection of instruments is available such as:

- energy instruments used for coagulation, cutting or dissection;
- grasping instruments;
- needle drivers that have a cutting blade integrated;
- retracting instruments;
- suction and irrigation probes;
- clip appliers, probe graspers and other specific instruments.

When replaced the instruments are automatically recognized and their type and function is displayed. The endowrist instruments are defined by their micro-articulation near the active tip, that gives them 7 degrees of freedom, that being the greatest possible motion around a joint, making these arms able to reach around, beyond and behind. Some studies were done on the endowrist particularly, approaching aspects like suture damage, grasping (Hirano et al., 2010; Teoh et al., 2017). The force applied can be set from the computer to deliver as much needed for the task at hand. In addition to the active instruments, a lightweight 3D HD camera is introduced inside the body for vision setup.
The vision cart stores components of the visual system and monitors that allow the team to watch the intervention. The new system has the TilePro feature that is a multi-image display that can show on screen simultaneous images of the surgical field and two other video sources like ultrasound or EKG (www.intuitivesurgical.com, 2018).

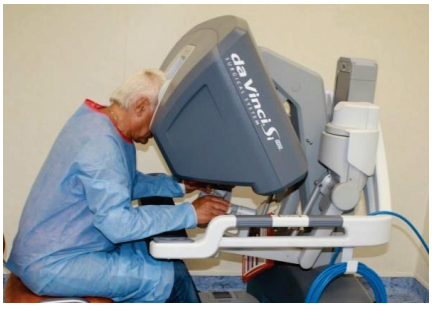

Figure 2. Da Vinci console

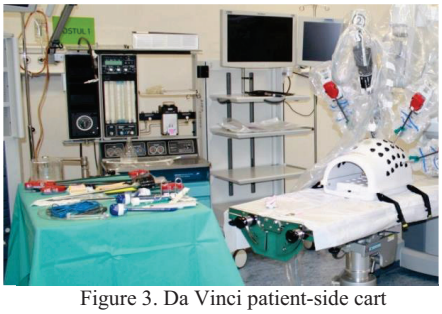
Figure 3. Da Vinci patient-side cart

Robotic surgery applications

Being performed on live animals just for experimental or training purposes at the moment, robotic surgery is still a field that exclusively defines human medicine (Gastrich et al., 2011).

Over the past 20 years, numerous articles were published mentioning the use of robotic surgery as an alternative to laparoscopic or open surgery or even as a unique, groundbreaking treatment, but the lack of sufficient data makes it impossible, at this moment, to establish its real utility (Al-Naami et al., 2013; Alemzadeh et al., 2016; Singh, 2011).

After reviewing the literature on robotic surgery applications, it can be said that the most frequent procedures belong to the following fields:

- Neurological surgery;
- Urological surgery;
- Gynecological surgery;
- Cardiothoracic surgery;
- Gastrointestinal surgery;
- General surgery.

In a short period of time, robotic interventions have been executed in numerous surgical fields, rapidly becoming a possibility for almost every medical specialty. The most cited procedures which can be performed with the assist of a robotic system are urological and gynecological.

Robotic surgery is an option for a significant number of urological procedures but its great success and groundbreaking techniques, that made the da Vinci system so popular, apply to the prostate followed by the kidneys and bladder (Yates et al., 2011).

The prostatectomy performed on patients diagnosed with prostate cancer is the most discussed procedure of its type (Orvieto and Patel, 2009). While there are a large number of studies that report the advantages of this method and implicitly the superiority of robot-assisted radical prostatectomy (RARP) when compared with the laparoscopic radical prostatectomy (LRP), other reviews conclude that the literature is limited and that the methodological quality is low, questioning the validity of the results that declare the robotic procedure superior to the laparoscopic one (Boggie et al., 2014; Box and Ahlering, 2008; Kang et al., 2010). Although the procedure's costs are high, the robotic radical prostatectomy tends to become the golden standard in treating patients with prostate cancer. Robots assist in oncological surgeries of the kidneys and bladder with a better outcome than in classical procedures. The most common robotic procedures done on kidneys are the partial nephrectomy and pyeloplasty, while for the bladder the method of choice for a patient with cancer is the robotic-assisted radical cystectomy (Benway et al., 2009; Bozzini et al., 2016; Dal Moro, 2017; Hubert et al., 2003; Kingo et al., 2016). Other urological interventions are performed such as ureteral reconstruction (Brandao et al., 2016).

The first cardiac surgery performed with a robotic system was in 1999 and since then the most challenging surgical interventions are being performed using the da Vinci system (Kim et al., 2018; Poffo et al., 2013).

The major types of robotic heart surgeries:

- Coronary artery bypass;
- Mitral valve repair or replacement;
- Tricuspid valve repair;
- Atrial septal defect repair;
- Patent foramen ovale repair;
- Removal of cardiac tumors.

Other thoracic procedures are experimentally done, such as robot-assisted pulmonary lobectomy (Lococo et al., 2014).

Regarding general surgery, complicated and demanding procedures such as pancreatectomy, Whipple surgery, liver resection for transplant have been done especially in the last 10 years (Baek and Kim, 2014; Lomanto, 2001; Panaro et al., 2011; Rashid et al., 2015; Tselios, 2013).

Robotic surgery in gynecology is growing at a fast pace, being used in the present to treat oncological pathologies and also fibroids, endometriosis, pelvic prolapse (Scandola et al., 2011).

In the fields of neurosurgery and orthopedics specially designed robots, with limited and precise functions are used, the da Vinci system, which can be used only for soft tissues, not being one of them (Beasley, 2012).

Training methods

One of the most challenging aspects of implementing a robotic surgical center is

training the surgeons. Being such a new specialization few regulations and training protocols exist (Brinkman et al., 2016; Hung et al., 2013). Most published articles and reviews state that a step by step training program should be applied.

A surgeon that participates in robotic surgery training has to follow several phases, such as:

- A didactic phase;
- Inanimate laboratory;
- Cadaveric laboratory;
- Animal laboratory;
- Operative observation and operating under supervision (Chitwood et al., 2001).

The first step of the learning process is gaining theoretical knowledge. The trainee needs to learn about the robot's functions, its uses and the possible technical problems that can occur during surgery. This step includes also learning how to properly position the patient, followed by correct port placement, both being of the utmost importance in assuring access to target organs. Online courses are available on basic robotic surgery concepts, offering information on robotic components and troubleshooting.

The second step in robotic surgery training is the so-called inanimate laboratory or skills laboratory. This part can include several training methods depending on the curriculum in place. Training exercises from the basic movement of the robotic arms to more complex procedures can be performed during this lab, but they are mainly used for learning how to control the instruments, adjust the camera and familiarize with the system.

Also designed for gaining hands-on skills, the virtual reality (VR) simulators are a great, cost-efficient learning tool. Currently, five VR simulators are available for robotic training:

- Robotic Surgical Simulator (RoSS);
- dV-Trainer;
- SEP Robot;
- Da Vinci Skills Simulator (Culligan et al., 2014);
- Robotex mentor (Schreuder et al., 2011).

These simulators have been upgraded over the years, offering now the possibility of learning specific procedures not only basic skills. The trainee watches a video of a specific intervention being performed while the robotic arms move accordingly with the surgical steps. After registering all the movements, the trainee can try and reproduce them (Bric et al., 2014; Hammound et al., 2008; Hart and Karthigasu, 2007; Liu and Curet, 2015; Beyer-Berjot and Aggarwal, 2013).

Apart from using synthetic dummies or inorganic models, surgeons can be trained using wet labs. The training material used for the wet labs is represented by animal or human cadavers, animal tissues and organs or live animal models. These models can be used for procedural training, enabling the surgeon to practice in the most realistic conditions. Cadaveric models are an excellent material to practice dissection, excision and other basic techniques, while the live animal models allow in addition, vascular control which can only be learned during wet labs. Laboratories that use live animal models are more expensive, harder to schedule and in some countries raise ethical or religion-related problems (Sridhar et al., 2017). Although dogs, sheep and other live animal models have been used, the most chosen animal model for surgical training sessions is the pig. Swine are considered a great model due to their similarities with human anatomy and physiology (Ganpule et al., 2015; Joseph et al., 2008). Surgeons should enroll to live animal labs as the last step of their training or near the ending of their learning curve (Fig. 4).

After gaining practical skills, the next step for a surgeon is observing live cases, starting with videos and continuing in the operating room. To finish their training and start performing robotic surgery, the trainee needs to be under direct supervision of an expert, a proctor at first and then a mentor, the latter allowing the trainee more opportunities to operate (Santok et al., 2016). Mentoring can be made easier by the mentoring console, which is a dual console with two modes: "swap" and "nudge", that allow the surgeons to operate simultaneously or to shift the control from one surgeon to another. There are studies that bring arguments in favor of surgeons undergoing laparoscopic training before learning robotic techniques (Abaza, 2009).

An important aspect of the training is the "learning curve", which represents the number of surgical procedures performed before a doctor has an acceptable surgical outcome, analyzing parameters such as blood loss,

complication rate and the conversion rate to open surgery (Hanly et al., 2004; Heemskerk et al., 2007; Hassan et al., 2015; Hayn et al., 2010; Lenihan et al., 2008). The learning curve for hysterectomies in considered 50 cases and for RARP is 40 cases.
A form of evaluation is also required to assess the technical skills of surgical trainees. Several methods are in the process of implementation at the moment, but often the Objective Structured Assessment of Technical Skills (OSATS) is used as a global rating scale (Niitsu et al., 2012).
More and more medical centers with training facilities, approved to teach residents and fellows, try to include robotic surgery into their programs, creating curriculum levels and regulations.

Figure 4. Training session for robotic surgery using a swine model.

Financial aspects

The greatest problem that medical centers face when considering to introduce a robotic system to their hospitals, is the cost. Robotic surgery is expensive due to the cost of equipment, maintenance and repair fees, due to the cost of additional surgical training and of an increased operating room setup time (Hussain et al., 2014). The da Vinci Surgical System, used for soft tissues surgery, being the main surgical robot, has costs per unit that range from 1 to 2.5 million dollars (Barbash and Glied, 2010). To this amount are added maintenance costs of over 100.000 $ per year. Every surgery depending on its complexity and duration brings additional costs of 2.000$ to 4.000$, which includes drapes and replacement tools. A cost analysis was made in Turkey and concluded that money can be saved if the time used in the preparation stage is shortened. In this study, the time before the actual intervention started, was divided into three intervals: first ending when the anesthesia was ready, the second one when the drapes were secured and the last one ended after the ports were introduced. All of these steps can be realized faster by properly trained professionals, reducing the costs (Zeybek et al., 2014).
This being the financial situation associated with robotic surgery, a cost-effectiveness analysis has to be done, weighing the potential benefits, such as lower complication rate, reduced hospital stay, smaller incision and disadvantages represented by the increased costs. There are not enough studies published to conclude if the robotic surgery procedures are superior to the laparoscopic ones and it can't be said if the increased costs bring real medical advantages (Davies, 2014).
Many authors consider that in the future, with increasing the competition between the robot manufacturers and with more trained surgeons being able to perform robotic surgery, the costs will be reduced. Another aspect of the prohibitive prices is the monopoly that the manufacturer of da Vinci robot has over the production of robotic parts. In the future, when this technology will lose its patent protection, cheaper components will be produced by other companies, robots becoming more affordable and accessible even for veterinary medicine (Mayhew, 2014).

Future of robotic surgery

With a short history of under 30 years, robotic surgical systems represent cutting-edge technology, being the most advanced surgical equipment on the market. Although they have numerous advantages, these systems can be further improved and redesigned, steadily evolving.
For now, telesurgery is possible only with the surgeon being in the proximity of the patient, but it is envisioned that wireless commands will be developed and the distance between the surgeon and the patient can significantly grow.

The da Vinci system is used with manually switching the instruments, but manufacturing an automated system will save time and will remove the need for an assistant to do this job. Another way to improve this system is integrating voice command that can control several functions, making the system even more automated. Surgeons suggest that robotic systems should have integrated diagnostic testing equipment like ultrasonography or small microscopes. In the following years, the surgical robots are expected to become smaller, with more sensors, better cameras, with new and revolutionary instruments. Besides the technical improvements and novelties envisioned for this systems, the surgical efficiency is expected to increase due to shorter operative time, once the surgeons gain more experience.

Surgical robots may change direction from being multifunction, proper for every procedure system to smaller, simpler, more task focused devices.

Robotic surgery faces some challenges concerning ethical and legal aspects, that have to be addressed in the future, such as:

- If a technical problem occurs during surgery it goes under malpractice liability or the blame goes to the technical team or manufacturer?
- If the robotic surgery offers a marginal benefit is it ethical to impose a financial burden on medical systems or the patients (Kumar Pal et al., 2011)?
- If telesurgery will become possible for longer distances, the regulation of which country will apply when surgery is performed, when the surgeon and the patient are in different countries?
- Should some training requirements be set in place?

Although robotic systems were build to address the limitations of laparoscopic surgery, they will push the boundaries of medicine by further extending human abilities, but not being able to take over for human surgeons in the near future.

CONCLUSIONS

Robotic surgery is described among minimally invasive techniques as revolutionary, with several advantages associated, such as reduced blood loss, less postoperative pain, shorter hospital stays, smaller incisions that bring cosmetic benefits. Besides the benefits brought to patients, robotic surgery seems to overcome the limitation of laparoscopic surgery, making it possible to reach areas inaccessible to conventional laparoscopic instruments, offering a 3D magnification of the surgical site, instruments that have articulation with 7 degrees of freedom and a specially designed console that brings a more comfortable setting to the surgeon. Having all these characteristics and improving surgical precision and dexterity, robotic surgery may seem a perfect choice, but numerous questions about this system remain to be answered.

Longer operative times and the expensive equipment make the cost of a robotic surgical intervention go higher than for a similar procedure performed laparoscopically. Almost every study to date highlights the need for more randomized studies to be conducted, in different settings to determine if the benefit of using a robotic system overcomes the cost of its implementation. Studies need to be run to evaluate the true benefits of robotic surgery, to demonstrate its superiority over laparoscopic procedures or to confirm their similar results.

It is suggested that nowadays some medical centers embraced robotic surgery to keep their status as a cutting-edge facility, because of its popularity, the apparently reduced morbidity, its potential use for groundbreaking procedures and the ability to conduct experimental studies with this technology. Although the cost slows the expanding of robotic systems, a couple thousand robots are used all over the world and with more studies started every day and a prediction of a diminished cost in the future, the reluctance that most surgeons and patients manifest will fade.

This field also faces challenges about vague regulations, about the fact that few residency centers offer robotic surgery training and that a small number of training facilities exist. These facilities need a second system to be used exclusively for training which implies huge additional costs.

Robotic surgery will be further improved, new instruments and accessories will be developed and it will become an important part of the

history of minimally invasive surgery once it will be settled when and for what type of procedure it will be best used, once all the questions about its efficacy, safety, cost-effectiveness and training requirements will be answered.

It is considered that will become an interest for veterinary surgery as well, as soon as it becomes more affordable and more applications become available. This prediction is supported by the fact that veterinarians already collaborate with human medicine specialists in training sessions that use live animals.

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SEROLOGICAL SURVEY OF CAPRINE ARTHRITIS-ENCEPHALITIS VIRUS INFECTION IN SIBIU COUNTY, ROMANIA

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Abstract

Caprine arthritis-encephalitis (CAE) is an economically important viral disease of goats, caused by a single-stranded RNA lentivirus belonging to the Retroviridae family, Orthoretrovirinae subfamily. CAE virus (CAEV) infection is widespread among dairy goats in most industrialized countries. Clinical disease in goats includes encephalitis in kids, chronic arthritis, inflammatory mastitis and progressive respiratory disease in adults. Transmission of CAEV occurs generally via colostrum and milk consumption, but horizontal transmission also contributes to disease spread. Most goats infected remain virus positive for life, generally asymptomatic and can develop CAE months to years later.

The aim of the study was to investigate the seroprevalence for CAEV infection in goat herds in 6 villages from Sibiu: Arpașu de Jos, Avrig, Cârțișoara, Laslea, Porumbacul de Jos, Șelimbăr. A number of 15,947 serum samples were collected over a 3 year period. All samples were analyzed using a commercial Agar Gel Immunodiffusion (AGID) test. The results have shown that the overall seroprevalence was higher 9.06% in the last year of the study than in previous two years 3.54% and 2.29%. The biggest difference was found in Laslea with 17 positive samples (1.63%) in the first year and 86 positive samples in the 3rd year (14.90%).

The result of the study provides useful information on animal health and herd management factors. Proper prevention and control measures should be taken in order to prevent infection spreading and economic losses due to CAEV infection in the coming future.

Key words*: CAE, AGID, seroprevalence.*

INTRODUCTION

Caprine arthritis-encephalitis (CAE) was first described in the United States by Cork et al., in 1974, while the causal virus was first isolated in the same country from the synovial fluid of an arthritic goat (Crawford et al., 1980). Caprine arthritis-encephalitis has emerged as a significant and costly disease of goats (Smith & Sherman, 2009).

CAE is produced by a single-stranded RNA lentivirus belonging to the family Retroviridae. The retroviridae consist of non-oncogenic viruses that produce multi-organ disease characterized by long incubation periods and persistent infection (Balbin & Mingala, 2017). In general, goats with CAE virus (CAEV) do not show clinical signs of infection and detection of specific antibodies is the only test that shows the infection. The development of a clinical disease may take several months to years, probably due to very low seroconversion (Balbin & Mingala, 2017; Kaba et al., 2009). The infection causes slow and persistent inflammatory diseases characterized by synovitis, polyarthritis, mastitis and pneumonia in adult goats and encephalomyelitis in kids (Kaba et al., 2009; Lofstedt, 2016). Monocyte/macrophage lineage is the main target for the virus. In these cells, virus replication is closely associated with the differentiation and maturation of monocytes to macrophages (Kaba et al., 2009).

CAEV is transmitted by direct contract via colostrum (lactogenic transmission) and by horizontal transmission through respiratory secretions (OIE, 2017). Live animal trade represents a major risk in the diffusion of CAEV from one herd to another (APHIS, 2007).

Infection with CAEV in goats from Romania has been reported (Gurău et al., 2015; Enache et al., 2017). The ideal method of confirming the diagnosis of caprine arthritis encephalitis is the combination of clinical and serological methods. Nevertheless serology is the most cost-efective method for diagnosing (Spînu & Șandru, 2017). Agar gel immunodiffusion test (AGID) and enzyme-linked immunosorbent assay (ELISA) are considered sufficiently reliable to be used in control programs. The control of CAE infection could be made by

serological methods (ELISA or AGID). Both tests are considered sufficiently reliable for use in control programs. AGID test is reported to be more specific but less sensitive than the ELISA (Lofstedt, 2016). PCR can become a detection test in rigorous eradication programs to determine the infectious status of animals that cannot be diagnosed with serological certainty (Spînu & Șandru, 2017).

There are no vaccines available for CAE (Reina et al., 2013). Control programs remain the only approach to avoid infection and most control programs focus on elimination of infected animals and their progeny (Minguijon et al., 2015; Perez et al., 2010).

The aim of the study was to investigate by AGID the serological prevalence of CAEV infection in goat herds from a semi-intensive goat raising area located in Sibiu county.

MATERIALS AND METHODS

A number of 15,947 serum samples were collected over a 3 year period and analyzed using a commercial Agar Gel Immunodiffusion (AGID) test. The samples were taken randomly from goat herds from 6 villages from Sibiu county: Arpașu de Jos, Avrig, Cârțișoara, Laslea, Porumbacul de Jos, Șelimbăr (Fig. 1).

All samples were analyzed using a commercial Agar Gel Immunodiffusion (AGID) test (Porquer Montpellier, France) according to the manufacturer's instructions.

The analysis of collected data was realized using commercially available spreadsheet software (Excel 2007).

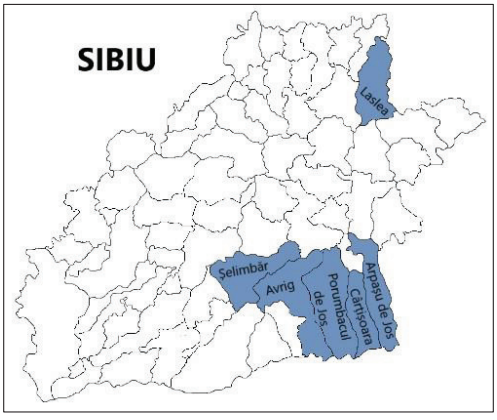

Figure 1: Assay-sampling from all 6 villages from Sibiu

RESULTS AND DISCUSSIONS

The distribution of the results obtained in all 6 villages is presented in table 1.

A total of 129/5621 (2.29%) serum samples were positive for CAEV in the AGID in the 1st year. Seropositive animals were present in 5/6 villages, indicating widespread infection. In the 2nd year the number of positive samples increased 275/7766 (3.54%).

The results have shown that the overall seroprevalence was 9.06% higher in the last year of the study than in previous two years in which values of 3.54% and 2.29% were recorded. The highest difference was found in Laslea with 17 positive samples (1.63%) in the first year and 86 positive samples in the 3rd year (14.90%).

Two main CAE developmental patterns were observed in the studied flocks. From year one to year three, in 50% of the locations, there was a steady, from 2 to 10 fold increase, of the positive animals (from 3.29 to 6.46% and 0.81 to 10.67% respectively), while in the other 50%, a sudden of 2 to 5 fold increase (3.32 to 8.09% and 0.0 to 5.82%, respectively), was noticed (Fig. 2).

These data supported the impact of differentiated raising systems' and biosecurity measures' implementation in various flocks. The movement of animals among dairy farms might increase the risk to introduce infected goats and cause higher seroprevalence in those farms.

At national level, the seroprevalence (38.46%) reported by Gurău et al. (2015) is much higher, due to the restricted area (Brăila County) of the analyses carried out by they authors.

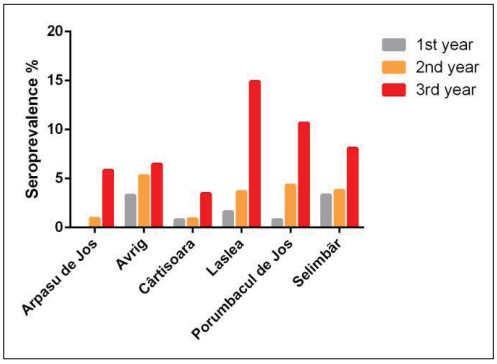

Figure 2: Comparison of the CAEV seropositive goats in all 6 villages from Sibiu

Table 1: Distribution of CAEV seropositive samples according to the herd origin

Villages	Results								
	1st year			2nd year			3rd year		
	Samples	+	%	Samples	+	%	Samples	+	%
Arpașu de Jos	341	0	0	1277	12	0.94	361	21	5.82
Avrig	1701	56	3.29	1622	86	5.30	526	34	6.46
Cârțișoara	254	2	0.79	337	3	0.89	116	4	3.45
Laslea	1042	17	1.63	2111	77	3.65	577	86	14.90
Porumbacul de Jos	866	7	0.81	994	43	4.33	300	32	10.67
Șelimbăr	1417	47	3.32	1425	54	3.79	680	55	8.09
TOTAL	**5621**	**129**	**2.29**	**7766**	**275**	**3.54**	**2560**	**232**	**9.06**

CONCLUSIONS

The obtained data provided useful information on the influence of herd health management factors in controlling the spreading of CAE in the selected flocks. Proper prevention and control measures must be designed to prevent further economic losses due to CAEV infection in the area.

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PARVOVIROSIS: A CASE REPORT AND A REVIEW OF LITERATURE

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Abstract

This paper aims to present a case report of three young Labrador puppies six months old, puppies living in three different homes, but belonging to the same litter in Cluj-Napoca. In addition to this this paper aims to review the literature regarding parvovirosis. Parvovirosis is an extremely contagious and fatal disease that occurs especially in young dogs, affecting the gastrointestinal tract and in more extreme cases it also affects the heart of the patients. Parvovirosis is produced by a virus of the Parvoviridae family, the Parvovirus genus, more exactly CPV-2. Preventable through vaccination, the newest cases have shown that even the best vaccines are ineffective, resulting in the apparition of the horrible disease. The diagnosis is quite simple to put, the clinical signs being extremely relevant. Additional tests may be used, helping with the assessment regarding the evolution of the disease. The prognosis of this disease depends of the virulence of the virus strain as well as the response of the organism to the treatment. However, it is also important that the treatment is started as soon as possible, because without it, the prognosis is fatal. These three young Labrador puppies are the living example that the vaccines used in them for the prevention of this fatal disease were not efficient. The disease first appeared in cats 1978, but since then the disease has appeared to dogs as well. Lately in Cluj there has been an explosion of cases, the virus attacked without considering the vaccine status of patients. This paper will present the outcome of the treatment in these particular cases, more exactly two males and one female, belonging to the same litter, all three being treated inside the Infectious Diseases Clinic of the Veterinary Medicine Faculty in Cluj Napoca.

Key words*: parvovirosis; dogs; infectious.*

INTRODUCTION

Parvovirosis is a infectious, highly contagious disease, which was first described in cats in 1978, but it had rapidly evolved and developed in dogs as well (Carmichael, 2005). This is one of the most important viruses that infect both the wild and domestic canids, being found worldwide. Parvovirus type 2-c (CPV-2) is a small non-enveloped virus, belongs to the *Parvoviridae* family and contains a single stranded DNA of about 5.2 kb in length (Miranda et al., 2015). The virus has a , non-enveloped icosahedral capsids (Xie & Chapman, 1996). The capsid contains 60 protein subunits of VP1 and VP2 (54–55 copies). These all share the same structure and are produced by the alternative splicing of viral mRNAs (Miranda et al.,2016).

The central core of the capsid is highly conserved that is composed of an eight-stranded, antiparallel β-barrel with flexible loops between the β-strands. These strands form the surface of the capsid which has a 22 Å spike on the threefold axes, a 15 Å deep canyon that surrounds the cylindrical structures at the fivefold axes, and as well as a 15 Å deep depression “dimple” at the twofold axes. Moreover the threefold axes are the most antigenic region of the capsid. They serve as a target for neutralizing antibodies (Agbandje et al., 1993).

Studies have shown that the disease occurs in 6–12 week-old pups; while younger dogs are generally protected from infection by maternally-derived immunity (Desario C., et al., 2005). Other studies have shown that puppies between 6 weeks and 6 months are more vulnerable to the disease (Prittie, 2004). In contrast with the literature findings, the oldest patient with parvovirosis was 12 months old.

Regarding the breed susceptibility, literature cites that certain black and tan colored dogs have a higher sensitivity to the disease. Rottweilers, Doberman Pinscher and Pitbull

terriers being some of them (Nelson et al. 1998). Another research quotes the Labrador Retriever and German Shepherd as two breeds that commonly develop parvovirosis (Nandi et al., 2010). Goddard and Leisewitz (2010) declare that male that are not neutered have twice the bigger risk of developing CPV, than the sexally intact females.

There are two ways of contamination, the direct one, that takes place through the fecal-oral route, and the indirect one through exposure to infected objects, clothes, people. It is extremely important to be noted that the fecal excretion will take place up to 4 weeks after the clinical sign have been ameliorated. The replication begins in the lymphoid tissue of the oropharynx, mesenteric lymph nodes and thymus. From here it is spread to the intestinal crypts of the small intestine via hematogenous spread (Amelia Goddard).

Diagnosis of CPV can be based on the clinical signs but literature cites the tests that can detect viral antigens by means of antibody based methods, because they are handy and available on the field. However, their sensitivity, has been proven inferior to molecular testing. An immunochromatographic (IC) test was compared to molecular techniques, and it was revealed that the sensitivity of the test did not exceed 50%, whereas the specificity was 100%. It was believed that these poor results were because the virus doesn't always shed in the feces or it is shed in low amounts (Desario et al., 2005).

Some studies have revealed that hemagglutination and virus isolation were tried as alternative techniques to diagnosing CPV but it is rather difficult and only well equipped laboratories are prepared for this.

Subsequently, several PCR assays were developed that displayed increased sensitivity and specificity in comparison with traditional methods (Marulappa et.al, 2009).

Treatment ideally also consists of crystalloid IV fluids and/or colloids, antiemetics and broad-spectrum antibiotic injections (Macintire et al, 2008).

Fluid therapy is extremely important, combating dehydration and electrolyte imbalance. The fluids are typically a mix of a sterile, balanced electrolyte solution, with an appropriate amount of B-complex vitamins, Haes. Some authors recommend enrofloxacin as a broad spectrum antibiotic but some of them not, because it affects the growing cartilage.

There have been some reports saying that oseltamivir cand reduce the severity of the disease and shorten the disease. This neuraminidase inhibitor may limit the virus ability to invade the intestinal crypt cells, decreasing the toxin production (Macintire, et al, 2006). Without the treatment CPV is fatal.

This is a preventable disease through vaccination and rigorous disinfection Ettinger et al 1995), Oh et al, 2006).

MATERIALS AND METHODS

Three Labrador Retrievers, 6 months old puppies, two males and one female, (Marco fig 1, Missy, fig. 2 and Spark fig. 3) presented in the fall of 2017 at the Emergency Hospital from the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca. They live in separate homes but they come from the same litter, all presented vomiting and haemorrhagic diarrhea and they were further redirected to the Infectious Diseases clinic. The health books shown that all three subjects were vaccinated with the vaccination scheme complete, the vaccines used are believed to be good and efficient. The last vaccine was 3 months before the signs developed. Internal deworming was as well done properly. Rapid snap tests were taken and all three came out as positive. Haematology revealed leukopenia in different stages for the three puppies. Coproparazitologic exam revealed that one of the puppies has Sarcocystis spp. After further questioning the owner we learned that the fed him raw meat. In Table 1 it is shown the medication treatment applied to the dogs, describing the action mechanism and the general use of them.

The treatment plan for all of the three patients varied on body weight and the dehydration degree. Parvovirosis being a viral disease, the only treatment that is appropriate is a symptomatic one. It aims to combat dehydration by stopping vomiting and diarrhea as well as strengthening the immune system through vitamin therapy. An important step in the process is general antibiotic therapy as well

as an anti-infectious medication. In addition to this autohemotherapy was done to all three puppies.

Fig. 1. Marco one of the Labradors (original)

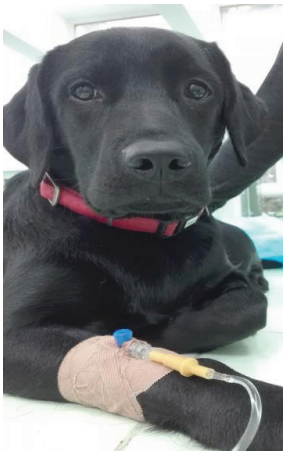

Fig. 2. Missy the female Labrador (original)

Fig. 3. Spark the third puppy (original)

Table. 1.Therapy medication, dosage and their action

Medication	Dosage	Action
Ringer Solution	Depends on the dehydration	It is used to supplement fluids and salts in the blood.
Duphalyte	25-50 ml/5 kg	Solution made from vitamins (B1, B6, B12, Nicotinamide, Dexpanthenol), Electrolytes (Ca, Mg, Cl) and Aminoacids (Arginine, Cisteine, Anhydrous dextrose)
Tetraspan	10-20 ml/kg	It promotes retention of the fluid in the vascular system through the exertion of oncotic pressure
Metronidazole	10-15 mg/kg	The mechanism is not entirely known but it appears that in anaerobic conditions it binds to DNA and causes cell death.
Pantoprazole	2 mg/kg (can be divided into two doses)	"Selective proton pump inhibitor", a medicine that reduces the amount of acid produced by the stomach.
Hyoscine Butylbromide	1 ml/10 kg	Is effective against spastic pain in the gastrointestinal organs, bile and urethral tract
C Vitamin	1 g/day	It stimulates tissue oxidation processes and has antitoxic and anti infectious action; helps maintain the integrity of the capillaries. It interferes with the healing of the lesions and the blood clotting. It increases the effectiveness of antimicrobial therapy.
Etamsylate	2 ml	Improves platelet adhesion, increases capillary resistance and reduces its permeability, shortening bleeding time and reducing blood loss.
Carbazochrome	2,5-5 ml	It is capable of stopping low-intensity bleeding.
Amoxicillin + clavulanic acid	8,75 mg/kg (once a day)	Broad spectrum antibiotic, eficient against both Gram negative and Gram positive bacteria
Maropitant	1 mg/kg	Inhibits vomiting reflex by blocking NK-1 in medullary vomiting centre.
Enteroguard	1 tablet/ 3 kg	Enteroguard M tablets are used to prevent and combat primary and secondary enteropathy produced by bacteria and protozoa
Eridiarom	1tablet/3 kg	Eridiarom reduces intestinal peristalsis without constipation; is astringent of the gastrointestinal mucosa, bacteriostatic, antiseptic, antihistaminic, antiparasitic and hypoglycemic.
No-Spa	2 mg/kg	Drotaverine is an antispasmodic agent whose action is based on significant inhibition of phosphodiesterase enzyme (PDE), responsible for AMP-cyclic hydrolysis (AMPc) in AMP, resulting in smooth muscle relaxation
Autohaemo-therapy	5 ml of the patient	Injected in the sc axillary region

RESULTS AND DISCUSSIONS

The treatment lasted between 8 days, each patient receiving medical care as needed, the longest treatment being in the Spark patient, a patient who was also diagnosed with Sarcocystosis. The patient Marco was the first of the brothers who showed appetite for food and water, consuming them without vomiting or without diarrhea. This happened on day 4 of treatment, the next day again vomiting. Treatment of this patient continued until day 6, when body temperature was normal, present appetite, consciousness and joy, consumed food without any problems, and the haematological examination revealed an excellent state of health. Missy the female patient has developed an easier form of the disease, which only vomits one time during the course of the disease. From the very beginning hematology revealed a moderate leukopenia compared to her siblings. This supports the literature that states that females are more resistant to this virus. Given that it contacted a mild form, recovery was shorter. The third patient, Spark suffered from a slightly increased form of hemorrhagic gastroenteritis due to the presence in the feces of the *Sarcocystis* parasite. The treatment of this patient lasted 8 days, but at the end of the patient, the patient was clinically healthy.

Dog owners have been informed throughout the treatment of the evolution of the cases, as well as the measures to be taken to prevent as far as possible the spread of the virus. They were familiar with disinfectants that have the ability to destroy the virus (ecocide, sodium hypochlorite).

It is important to remember that dogs that pass through parvovirosis and survive, remain bearers and eliminators of the virus for a period of time that may vary from week to month.

The prognosis of this disease is generally reserved. For these cases, the prognosis was favorable because they were already vaccinated, being more or less immunized, as well as the fact that they were presented to the doctor as soon as they discovered that their puppies feel ill. Samples from each dog were taken for further analysis, regarding the strain producing parvovirosis.

CONCLUSIONS

Parovirosis is a worldwide spread infectious disease that affects young dogs and it can be fatal without treatment.

It seems like even if the dogs were vaccinated and well taken care of they still developed the disease, lighter forms of it, all thanks to the immunity the vaccination conferred them. It is not known why they contracted the disease, giving that they were immunized. This remains still unclear, but there are steps to solve the puzzle. Parvovirosis is a fairly studied disease, but it seems that the strains are very powerful and there are still so many aspects unknown.

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RESEARCHES REGARDING THE CENTRAL VENOUS CATHETER MANAGEMENT IN DOGS UNDERGOING HEMODIALYSIS

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Abstract

Central venous catheter associated infections are the major cause of morbidity and mortality in patients undergoing hemodialysis. Lockings with antibiotics associated with heparin are used for both prophylaxis of infections and central venous catheter longevity. The present study was conducted on 20 dogs treated in the Hemodialysis Clinic from the Faculty of Veterinary Medicine Bucharest. 10 dogs received a locking with 2500 IU/ml heparin combined with vancomycin 2.5 mg/ml and 10 had locking with 5000 IU/ml heparin. Locking solutions were maintained for 24 hours in each patient. Local reactions were observed on the jugular vein, and the central venous catheter function was evaluated in each patient. Catheters were maintained at least 10 days. There were no local reactions in the batch with vancomycin and heparin locking. In the heparin locking batch, local reactions occurred in three subjects on the third day, and central venous catheter functioning progressively altered until day 10. Locking with heparin and vancomycin are an effective alternative, the catheter having a longer maintenance period. Combining an antibiotic with heparin provides superior results to prevent catheter-related infections.

Key words: *hemodialysis, catheter, infection, heparin, antibiotic.*

INTRODUCTION

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, and is the primary element in the therapy of patients with acute or chronic renal failure undergoing hemodialysis (HD). Vascular access is the most important basic requirement of successful extracorporeal renal replacement therapy (ERRT) and an adequately functioning hemodialysis catheter allows for smooth and efficient patient management. Catheter manipulations maneuvers disturb the fibrinolytic system, triggering a cascade of coagulation and inflammation at this level. Finally, if the endogenous fibrinolytic system has the ability to excess coagulation, thrombosis occurs in the catheter.

Central venous catheter associated infections is a major cause of morbidity and mortality in patients undergoing hemodialysis regardless of the duration of therapy.

A standard therapy management of hemodialysis catheter-related infections and bacteremia management involves both catheter replacement and systemic antibiotics therapy. Therefore, lockings of antibiotics associated with heparin are used for the infections prophylaxis and the longevity of the central venous catheter.

Since the antibiotic does not penetrate into the body and remains strictly into the catheter, its toxicity is not of concern for the renal function.

Therefore the purpose of this study is driven by the need to maintain central venous catheter perfectly functional for a longer period of time, given the purpose for which it is used (hemodialysis) and to standardize a method as better locking catheter.

MATERIALS AND METHODS

Local reactions were observed in the jugular vein area, and the central venous catheter function was evaluated in each patient. For each patient, the catheters were maintained for at least 10 days.

In the 5000 I.U./ml sodium heparin locking batch, local reactions (hyperemia or inflammation, or both) occurred in 6 patients from day 3 and the catheter function progressively altered.

In the 2500 I.U./ml sodium heparin combined with 2.5 mg/ml vancomycin locking batch,

there were no local reactions and the catheter function has been kept constant in the proper parameters throughout its use.

RESULTS AND DISCUSSIONS

Local reactions were observed in the jugular vein area, and the central venous catheter function was evaluated in each patient. For each patient, the catheters were maintained for at least 10 days.

In the 5000 I.U./ml sodium heparin locking batch, local reactions (hyperemia or inflamemation, or both) occurred in 6 patients from day 3 and the catheter function progressively altered.

In the 2500 I.U./ml sodium heparin combined with 2.5 mg/ml vancomycin locking batch, there were no local reactions and the catheter function has been kept constant in the proper parameters throughout its use.

Tabel 1. Representation of the catheter function and local reactions occurrence for days one to five in the batch with 5000 I.U./ml sodium heparin locking

Crt. No.	PATIENT Breed, Age, Sex.	DAY 1 Local reaction (R) / Catheter function (F)	DAY 2 Local reaction (R) / Catheter function (F)	DAY 3 Local reaction (R) / Catheter function (F)	DAY 4 Local reaction (R) / Catheter function (F)	DAY 5 Local reaction (R) / Catheter function (F)
1.	HUMPHREY, BEAGLE, 13 YO, M	R - - - F + + +	R - - F + + +	R + F + +	R + F + +	R + F + +
2.	REX, GERMAN SHEPHERD, 9 YO, M	R - - - F + + +	R - - - F + + +	R + F + +	R + F + +	R + F + +
3.	PUPICEL, SHAR-PEI, 5 YO, M	R - - - F + + +	R - - F + +	R + F + +	R + F + +	R + F + +
4.	RICKY, ROTTWEILER, 7 YO, M	R - - - F + + +	R - - F + + +	R + F + +	R + F + +	R + F + +
5.	ADOLF, GOLDEN RETRIEVER, 13 YO, M	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - F + + +	R - - F + + +
6.	NERO, LABRADOR, 6 YO, M	R - - - F + + +	R - - F + + +	R + F + + +	R + F + +	R + F + +
7.	BARCCA, GERMAN SHEPHERD, 7 YO, F	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +
8.	DELTA, CANE CORSO, 5 YO, F	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +
9.	NORI, GOLDEN MIXED BREED, 8 YO, M	R - - - F + + +	R - F + + +	R + F + +	R + F + +	R + F + +
10.	CLEO, GERMAN SHEPHERD, 2 YO, F	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +

Tabel 2. Representation of the catheter function and local reactions occurrence for days six to 10 in the batch with 5000 I.U./ml sodium heparin locking

Crt. No.	PATIENT Breed, Age, Sex.	DAY 6 Local reaction (R) / Catheter function (F)	DAY 7 Local reaction (R) / Catheter function (F)	DAY 8 Local reaction (R) / Catheter function (F)	DAY 9 Local reaction (R) / Catheter function (F)	DAY 10 Local reaction (R) / Catheter function (F)
1.	HUMPHREY, BEAGLE, 13 YO, M	R + F +	R + F +	R + + F +	R + + F +	R + + + F -
2.	REX, GERMAN SHEPHERD, 9 YO, M	R + F + +	R + + F +	R + + F +	R + + F +	R + + + F -
3.	PUPICEL, SHAR-PEI, 5 YO, M	R + F + +	R + F + +	R + F + +	R + + F + +	R + + F +
4.	RICKY, ROTTWEILER, 7 YO, M	R + F + +	R + F + +	R + F + +	R + F + +	R + + F + +
5.	ADOLF, GOLDEN RETRIEVER, 13 YO, M	R - - F + + +	R - - F + +	R - - F + +	R - F + +	R - F + +
6.	NERO, LABRADOR, 6 YO, M	R + + F + +	R + + F +	R + F -	R + + F - -	R + + F - -
7.	BARCCA, GERMAN SHEPHERD, 7 YO, F	R - - - F + + +	R - - - F + + +	R - - F + + +	R - - F + + +	R - - F + + +
8.	DELTA, CANE CORSO, 5 YO, F	R - - F + + +	R - - F + + +	R - - F + +	R - - F + +	R - - F + +
9.	NORI, GOLDEN MIXED BREED, 8 YO, M	R + F +	R + F - -	R + + F - -	R + + F - - -	R + + + F - - -
10.	CLEO, GERMAN SHEPHERD, 2 YO, F	R - - - F + + +	R - - - F + + +	R - - F + + +	R - - F + +	R - - F + +

Table 3. Representation of the catheter function and local reactions occurrence for days one to five in the batch with sodium heparin and vancomycin locking

Crt. No.	PATIENT Breed, Age, Sex.	DAY 1 Local reaction (R) / Catheter function (F)	DAY 2 Local reaction (R) / Catheter function (F)	DAY 3 Local reaction (R) / Catheter function (F)	DAY 4 Local reaction (R) / Catheter function (F)	DAY 5 Local reaction (R) / Catheter function (F)
1.	PEPE, ABRUZZI SHEPHERD, 5 YO, M	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +
2.	DARIUS, MIXED BREED, 7 YO, M	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +
3.	MURPHY, SHAR-PEI, 5 YO, M	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +
4.	FOXY, CHOW CHOW, 8 YO, F	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +
5.	AKSEL, GOLDEN RETRIEVER, 6 YO, M	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +
6.	DONNA, MIXED BREED, 2 YO, F	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +
7.	MAX, PITTBULL, 3 YO, M	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +
8.	MARK, AMSTAFF, 2 YO, M	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + +	R - - - F + +
9.	BELLA, MIXED BREED, 12 YO, F	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +	R - - - F + + +

Table 4. Representation of the catheter function and local reactions occurrence for days five to 10 in the batch with sodium heparin and vancomycin locking

Crt. No.	*PATIENT Breed, Age, Sex.*	*DAY 6 Local reaction (R) Catheter function (F)*	*DAY 7 Local reaction (R) Catheter function (F)*	*DAY 8 Local reaction (R) Catheter function (F)*	*DAY 9 Local reaction (R) Catheter function (F)*	*DAY 10 Local reaction (R) Catheter function (F)*
1.	*PEPE, ABRUZZI SHEPHERD, 5 YO, M*	*R - - - F + + +*	*R - - - F + + +*	*R - - - F + + +*	*R - - - F + + +*	*R - - - F + + +*
2.	*DARIUS, MIXED BREED, 7 YO, M*	*R - - - F + + +*	*R - - - F + + +*	*R - - - F + + +*	*R - - - F + + +*	*R - - - F + + +*
3.	*MURPHY, SHAR-PEI, 5 YO, M*	*R - - - F + + +*	*R - - - F + + +*	*R - - - F + + +*	*R - - - F + + +*	*R - - - F + + +*
4.	*FOXY, CHOW CHOW, 8 YO, F*	*R - - - F + + +*	*R - - - F + + +*	*R - - - F + + +*	*R - - - F + + +*	*R - - - F + + +*
5.	*AKSEL, GOLDEN RETRIEVER, 6 YO, M*	*R - - - F + + +*	*R - - - F + + +*	*R - - - F + +*	*R - - - F + +*	*R - - - F + +*
6.	*DONNA, MIXED BREED, 2 YO, F*	*R - - - F + + +*	*R - - - F + + +*	*R - - - F + + +*	*R - - - F + + +*	*R - - - F + + +*
7.	*MAX, PITTBULL, 3 YO, M*	*R - - - F + + +*	*R - - - F + +*	*R - - - F + +*	*R - - - F + +*	*R - - - F +*
8.	*MARK, AMSTAFF, 2 YO, M*	*R - - - F + +*	*R - - - F + +*	*R - - - F + +*	*R - - - F + +*	*R - - - F + +*
9.	*BELLA, MIXED BREED, 12 YO, F*	*R - - - F + + +*	*R - - - F + + +*	*R - - - F + +*	*R - - - F + +*	*R - - - F + +*
10.	*LARA, LABRADOR, 9 YO, F*	*R - - - F + + +*	*R - - - F + + +*	*R - - - F + + +*	*R - - - F + +*	*R - - - F + +*

Legend:

R - moderate local hyperemia;
R - - light local hyperemia;
R - - - absent local hyperemia;
R + severe local hyperemia;
R + + severe local hyperemia and the occurrence of local inflammation;
R + + + severe local hyperemia and the occurrence of local infection;
F - catheter function in only 2 - 3 patient position;
F - - catheter function in only 1 - 2 patient position;
F - - - catheter function in only 1 patient position;
F + hard catheter function;
F + + mild catheter function;
F + + + perfect catheter function.

The picture (Figure 1) was obtained after the necropsy examination at the request and with the consent of the owner, by the benevolence of the Pathological Anatomy Discipline of the Faculty of Veterinary Medicine in Bucharest.

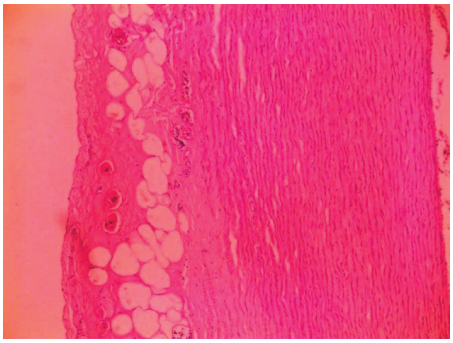

Figure. 1. The histological aspect of the transverse section through the cranial vena cava at the incidence with the catheter tip in a patient undergoing hemodialysis, whom died from cardiopulmonary arrest 7 days after central venous catheter assembly

CONCLUSIONS

The heparin and vancomycin locking is a better alternative, patients being protected from various local inflammatory infections or reactions, and the catheter has a longer duration of operation to complete hemodialysis therapy. Combining an antibiotic with heparin provides an alternative to stop infections and inflamemation associated with the central venous catheter.

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POSTOPERATIVE ANALGESIC MANAGEMENT OF GERIATRIC DOGS THAT UNDERWENT SOFT TISSUE SURGERY

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Abstract
This study was performed in order to evaluate the postoperative analgesic management of geriatric dogs during the first 12 hours after the soft tissue surgery. The study was conducted on sixty dogs, aged between 8-16 years, that were presented at the Faculty of Veterinary Medicine of Bucharest for soft tissue surgery procedures, between August - November 2017. Patients were divided in four groups (15 dogs /group) assigned to a different analgesic management that we intended to evaluate (Group 1- Tramadol, Group 2- Lidocaine in constant rate infusion, Group 3- Acetaminophen, Group 4- Ketamine+Lidocaine). Premedication was made with Midazolam 0.2 mg/kg and Butorphanol 0.2 mg/kg, injected intramuscularly (IM). Induction was obtained with Propofol 4-6 mg/kg intravenously. All patients were intubated, and maintenance was performed with Isoflurane. All patients were evaluated after the procedures using the Glasgow Composite Pain Scale (GCPS). During the evaluation period, the best results were recorded in groups 2 and 3 which were given Lidocaine and Lidocaine+Ketamine in a constant rate infusion with a pain scale of 2/24, compared with lower results in groups 1 and 4 with a GCPS of 5/24, where additional analgesic medication was needed.

Key words: *analgesia, geriatric patient, Glasgow Composite Pain Scale.*

INTRODUCTION

Nowadays pain is described as a complex, multi-dimensional experience involving sensory and affective components. (Lumb & Jones, 5th Edition), being described by the International Association for the Study of Pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (A Arntz, L Claassens, 2004).

At its simplest, pain can be classified as acute or chronic and according to the postoperative situation, it can be the most intense in the first 24 hours after surgery, declining gradually over time (Mathews K, et al., 2014). Acute pain, for exemple, can take different forms, depending on its severity, from mild to agonizing. Furthermore pain can also be divided into somatic pain, which arises in the skin (superficial) or in more profound tissues, like muscles, bones or joints (deep pain) and visceral pain (Robertson, 2002; Joshi and Gebhart, 2000). Regarding the causes of pain, it has been shown that pain can be produced particularly by tissue or nerve damage, inflammatory processes, viral infections or demyelination being characterised by pain hypersensitivity (Vinuela-Fernandez et al. 2007). It`s important to notice that patients, during and in the postoperative period suffer different changes and unpleasant events like pain, reduced organ function and prolonged hospitalisation (Kehlet H, Holte K. 2001). Therefore, the administration of an adequate postoperative analgesia is mandatory. Once noxious stimuli are formed, the peripheral nociceptors are activated, increasing the neuronal excitability, with a sensitization in the peripheral and central areas (Woolf C, Chong M., 1993). For a better understading, it`s important to know that the noxious stimulus is mediated by a nociceptive sensory system. Nociceptors represent the free endings of primary sensory neurons. The primary afferent nerve fibers that carry information from these free nerve endings to their central location consist of two main types: unmyelinated C fibers and myelinated A delta fibers. Activation of specific receptors and ion channels (present in most tissues and organs) in peripheral unmyelinated nerve endings by chemical, mechanical, or thermal stimuli causes the initiation of action potentials that propagate the

stimulus along the axons of primary afferent nerve fibers to synaptic sites in the dorsal horn of the spinal cord. This triggers the release of neurotransmitters, including glutamate and substance P, which activate neurons located in the spinal cord. (Perl ER., 2007).

Postoperative analgesia is required all the time, no matter what type of surgery was conducted, allowing a better recovery of the animal and a quicker return to its physiological normality. However, patients hospitalized after an orthopedic surgery may require different classes of drugs compared to the ones that underwent a soft tissue surgery. Analgesia protocols can include classes of drugs like: nonsteroidal anti-inflammatory drugs, local anesthetics, dissociative agents, acetaminophen. Opioids are another class of drugs, offering good sedation and mild to very good analgesia. Tramadol, a drug recommended in both human and veterinary medicine, is considered an opioid-like drug, being a weak μ-receptor which can promote analgesia by 3 different ways: 1. Its active metabolite, O-desmethyltramadol, which has analgesic properties, but comparing with humans, animals are not able to produce this metabolite in the same amount, therefore the analgesia seems to be weak ; 2. creating a binding with its enantiomer to μ-opioid receptors or due to inhibition of the reuptake of norepinephrine and serotonine (Giorgi et al., 2009; DeRossi et al., 2013). This drug can be used for the treatment of moderate to severe pain, acute or cronic at a bolus dose of 1-2 mg/kg. Depending of the severity of pain, the dose can reach 8 mg/kg. (Costea R., 2017).

Lidocaine is a local anesthetic agent which belongs to amide group (Feary et al. 2005) used for regional anesthesia or systemic analgesia (Costea R., 2017). Its benefits in veterinary medicine is widespread, acting like an antiarrythmic drug, especially in the treatment of ventricular tachycardia. Lidocaine has also an anti-inflammatory, anti-endotoxic effect, being a prokinetic agent (Bettschart-Wolfensberger & Larenza 2007; Enderle et al. 2008; Dzikiti 2013). Systemically administration offers analgesia for different types of pain (Ness, T. J., 2000; Smith, L. J. et al., 2002). This drug has a short life, about 60-90 minutes, compared with other local anesthetics, therefore is recommended its use as a constant rate infusion (CRI- 0.5-2 mg/kg/h), after the administration of a loading dose of 1-2 mg/kg i.v. Has been shown that using this administration technique the anesthetist can achieve an effective plasma concentration for systemic analgesia (Bettschart-Wolfensberger & Larenza 2007; Ringer et al. 2007). Smith et al. (2014), suggested that an intraoperative CRI of lidocaine provided postoperative analgesia similar to morphine CRI after ophthalmologic surgery in dogs, when given a loading dose (1 mg/kg) followed by a CRI (25 μg/kg/ minute).

Ketamine is a dissociative drug, commonly used in both human and veterinary anaesthesia (Noemi R. et al., 2017), being a non-competitive antagonist of the N-methyl D-aspartate (NMDA) receptors that may prevent central sensitization and cumulative depolarization from occurring (Pozzi et al. 2006). Besides its anesthetic effect, lower sub-anesthetic doses have been administrated as an adjunctive analgesic agent in dogs (Slingsby & Waterman-Pearson 2000; Wagner et al. 2002; Sarrau et al. 2007). Most of the authors recommended first the administration of a loading dose of 0.15–0.7 mg/kg^{-1}) followed by a CRI (2–10 μg/kg^{-1}/minute^{-1}) (Wagner et al. 2002; Sarrau et al. 2007; Costea R., 2017). Due to the side effects, also, other authors consider that ketamine might be used as part of a multimodal analgesic approach but not as a sole method of providing pain relief in dogs after surgery (Wagner et al. 2002; Sarrau et al. 2007).

Acetaminophen is considered a safe analgesic and antipyretic drug at therapeutic doses for dogs, however an overdose can induce hepatotoxicity, being the one of the major causes of acute hepatic failure (Chun LJ. Et al, 2009).

MATERIALS AND METHODS

Our study was conducted in the Clinic of the Faculty of Veterinary Medicine Bucharest, from August 2017 to November 2017, on sixty geriatric patients. Breeds enrolled included various mixed and pure breed dogs with age between 8-16 years old and body weight between 5-45 kg. The patients were anesthetized for various soft tissue surgical

procedures: splenectomy, mammectomy, ovariohysterectomy. Physical examination, complete blood exams were also taken into account. Following paraclinical examination, four (4) groups were formed depending on the analgesic drugs that we intended to use. Each patient was considered an adequate candidate for anesthesia and American Society of Anesthesiologist (ASA) scores were recorded for each animal. Dogs with an ASA score of II or III were included in the study.

For each group, premedication was made with butorphanol 0.2 mg/kg and midazolam 0.2 mg/kg administered intramuscularly (IM). Anesthesia was induced with Propofol (4-6 mg/kg IV). Spontaneous or intermittent positive-pressure ventilation (IPPV) were maintained by the use of a volume-cycled ventilator delivering 12-15 breaths/minute to achieve a target end-tidal CO_2 of 35-45 mm/Hg. Oxygen flow was initially delivered at 2 L/min with the vaporizer set to achieve an end-tidal concentration C% of 2.0% isoflurane within 10 minutes of induction. After the target concentration was achieved, oxygen flow was decreased to (500+10/kg) L/min, and isoflurane was constantly maintained at 1.5 vol. % in all cases.

Crystalloid solutions were administered at a rate of 3-5 ml/kg/h IV throughout anesthesia. ECG, heart rate, $etCo_2$, SpO_2 and esophageal temperature were monitored. Temperature was maintained between 37ºC-38 ºC by using a warm electrical blanket.

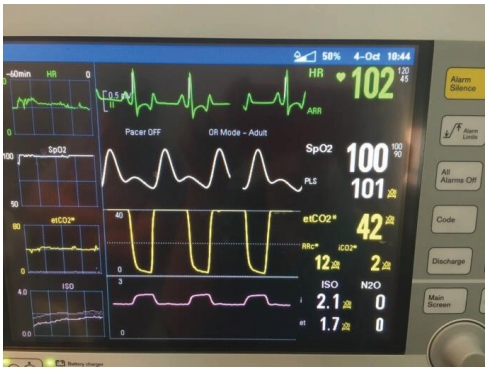

Fig. 1. Patient monitoring

At the end of the surgery the isoflurane was switched off and the residual anesthetic was flushed from the breathing circuit. Patients were extubated when they began to breathe spontaneously. The patients were taken into the ICU department where they received, according to the group classification: tramadol 2 mg/kg IV every 12 hours, patients from Group 1, Group 2 a CRI with Lidocaine (1 mg/kg IV ``loading dose`` then 0.025 mg/kg/min), acetaminophen 10 mg/kg every 12 hours was given to Group 3 and for the 4th group, a mixed Lidocaine and Ketamine in a CRI was administered (Lidocaine 1 mg/kg ``loading dose then 0.025 mg/kg/min + Ketamine 0.2-2 mg/kg/h).

All patients were evaluated using the Glasgow Composite Pain Scale (GCPS). Behavior categories used to assess pain included vocalization, attention to wound area, mobility, response to touch, demeanor and posture/activity. A categorical score was assigned within each behavior category based on the severity of the behavior or the response observed (Table 1). Potential cumulative pain scores ranged from 0 (least painful) to 23 (most painful). To ensure interpretative consistency, a single person was trained in evaluating the dogs for pain. The person was blinded to treatment. The person first observed the dog`s behavior from a distance so as not to disturb the dog, then the assessor increased his interaction with the dog, including manipulation of the surgical site and removing the dog from the cage to allow the dog to move around.

Table 1. Glasgow Composite Pain Scale (GCPS)

Behavior Category	Score	Definition
Vocalization	0	Quiet
	1	Whimpering or crying
	2	Groaning
	3	Screaming
Attention	0	Ignoring
	1	Looking
	2	Rubbing
	3	Chewing
Mobility	0	Normal
	1	Lame
	2	Slow or reluctant
	3	Stiff
	4	Refuses to move
Response to touch	0	Do nothing
	1	Looks around
	2	Flinch
	3	Growl or guard area
	4	Snap
	5	Cry

Demeanor	0	Happy and content and bouncy
	1	Quiet
	2	Indifferent or nonresponsive to surroundings
	3	Nervous, anxious or fearful
	4	Depressed or nonresponsive to stimulation
Posture/ activity	0	Comfortable
	1	Unsettled

Based on the dogs' response, each of the six behavior categories were scored. Records of the GCPS for each patient were assessed at 15 min., 30 min., 45 min., 1, 2, 6 and 12 hours after the analgesic drug was given. The results for the four groups were compared and analyzed according to the protocol`s effect at 12 hours after the first administration.

All dogs were observed for adverse reactions following pain medication therapy. Adverse reactions were characterized as minor if they were self-limiting and did not require additional therapy. Minor reactions included sedation, dysphoria, salivation and loss of appetite.

RESULTS AND DISCUSSION

Four treatment groups were created with a number of 15 patients in each group and a total of 60 dogs.

Breeds of dogs included mixed breed dogs (n=26), Labrador Retrievers (n=11), Golden Retrievers (n=6), German Shepherd Dog (n=4), Bichon (n=10), West Highland Terrier (n=3).

The analgesic protocols were well tolerated throughout the study period. Minor adverse effects occurred in 3/15 (20%) dogs from Group 3 which had a Lidocaine + Ketamine medication therapy, represented by salivation and vocalization versus 0 adverse effects reported in the other three groups. Overall 5/60 (8.33%) dogs required additional rescue analgesic therapy based on their pain scores. This included 3 dogs from Group 1 treated with tramadol, and 2 dogs from Group 4 treated with acetaminophen.

During the evaluation period, the best results were recorded in groups 2 and 3 which were given lidocaine and lidocaine+ketamine in a constant rate infusion with a pain scale of 2/24, compared with a poor result in groups 1 and 4 with a Glasgow pain score of 5/24, where additional analgesic medication was needed.

Table 2. Adverse effects and additional rescue doses of drugs

	Nr.	Adverse effects of the analgesic drugs	Additional rescue doses
Group 1 Tramadol	15	0	3
Group 2 Lidocaine	15	0	0
Group 3 Lidocaina+ Ketamina	15	3 salivation vocalization	0
Group 4 Acetaminophen	15	0	2

Analgesic drugs have been widely used for postoperative pain management in dogs (Wagner et al., 2002; Steagall et al., 2006; Lin et al., 2008; Uilenreef et al. 2008; Ortega & Cruz, 2011; Columbano et al., 2012). In this study, we compared the effects of acetaminophen, tramadol, lidocaine, lidocaine and ketamine administered postoperatively as analgesics for dogs that underwent soft tissue surgeries. We found a difference in clinical analgesic effects of acetaminophen and tramadol compared with Lidocaine and Lidocaine/Ketamine constant rate infusion.

A Glasgow Composite Measure Pain Scores through the study period were different between groups 1 and 4 compared to groups 2 and 3.

Tabel 3. Evaluation of GCPS at 12 hours after drug administration

	G 1	G 2	G 3	G 4
Vocalization	1	1	0	1
Attention to wound	0	0	0	0
Mobility	2	1	1	2
Response to touch	1	0	0	1
Demeanor	1	0	0	1
Posture	0	0	0	0

G1-Tramadol, G2- Lidocaine CRI, G3- Lidocaine+Ketamine CRI, G4- Acetaminophen

According to our study this may represent the low capacity of tramadol and acetaminophen to

produce acceptable levels of analgesia in the immediate postoperative period, when used in singular analgesic protocols.

CONCLUSIONS

This study represents a clinical investigation of acetaminophen, tramadol, lidocaine and lidocaine/ketamine analgesia in geriatric dogs.
Based on this study, the analgesic effect of tramadol and acetaminophen at the doses of 2 mg/kg iv respectively 10 mg/kg iv were not satisfactory compared with the analgesic effect given by the continuous infusions of Lidocaine at 0.025 mg/kg/min with a loading dose of 1 mg/kg iv. and Lidocaine (1 mg/kg loading dose then 0.025 mg/kg/min CRI) +Ketamine (0.2-2 mg/kg/h CRI).
Some adverse reactions were recorded after administration of Ketamine+Lidocaine, like vocalization and salivation, therefore we recommend the use of Ketamine with another analgesic drug that also has sedative effect.
A multimodal analgesia it is considered to be effective in reducing postoperative pain after soft tissue procedures.

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A REVIEW OF NEUROLOGICAL EXAMINATION-DIFFERENTIAL DIAGNOSIS FOR INTRACRANIAL DISEASES IN CATS AND DOGS

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Abstract

This review paper aimed to gather together the most relevant findings concerning the neurological examination and the localization of the lesion in the central nervous system. Clinical signs are specific depending on the regions of the brain involved (forebrain, brain stem, cerebellum or vestibular apparatus). In order to obtain and correlate these signs to a certain localization of the lesion, it is necessary that some steps be strictly followed when examining the animal. The first step is to obtain the full medical history of the patient by discussing with the owner. The physician must then perform the physical examination, followed by a complete and correct neurological examination. Neurological signs can be associated to a certain region of the brain. The mental status is evaluated first, followed by the behavior and the way the animal interacts with the environment, the postural reactions (head, body, limbs) and the gait, cranial nerves, proprioception, spinal reflexes, panniculus and perineal reflexes. When the examination is complete, all findings are taken into consideration and an accurate neuroanatomic diagnosis is established.

Key words*: neuroanatomic diagnosis, forebrain, brain stem, cerebellum, vestibular system.*

INTRODUCTION

In our country, although veterinary neurology is at a starting point, the number of patients with neurological deficits is increasing. Considering this situation, study and interpretation of the available scientific articles is essential in order to develop new clinical skills and to adjust the neurological examination protocol so it can suit our cases.

In order to establish an accurate etiological diagnosis, proper interpretation of neurological deficits and precise neuroanatomical localization are essential.

Diseases that affect one of the four regions of the brain (forebrain, brain stem, cerebellum or vestibular apparatus) are accompanied by a set of clinical features that are specific to each one of them. For the clinicians, the real challenge is to make a differential diagnosis between a systemic, orthopaedic, cardiovascular and neurological disease.

Sometimes clinical signs can indicate a lesion that affects the nervous system, but the etiological diagnosis requires a series of additional investigations in order to be established. In that case, an anatomic diagnosis helps the physician choose the most sensitive investigations for the suspected area.

This review briefly discusses the steps that have to be followed in order to localize an intracranial nervous system lesion.

MATERIALS AND METHOD

In order to make a selection of the most relevant articles for the debated subject, we searched in the veterinary scientific databases Pubmed and ScienceDirect using the keywords: neurologic examination, intracranial lesions, brain disease, vestibular disease. Considering the fact that veterinary neurology is a field that has developed especially in the last decades, the written articles on this subject are fewer compared to other areas. We have found about 40 articles, from which we have selected those who were written later than 2009. The provided information was compared with the information available in the reference books of neurology. The common points and differences which have resulted from this analysis are presented in the following sections.

RESULTS AND DISCUSSIONS

The neurologic examination is preceded by anamnesis and a complete and correct physical examination.

The anamnesis is a very important part of the evaluation and should include signalment of the patient and chief concern (the reason why the owner is presenting the pet to the veterinarian).
Patient's age, breed and sex are factors that helps the clinician focus on a certain pathology. For example, congenital or development diseases occur in the young, whereas neoplasia and degenerative disorders are more common in older patients (Thomas, 2010).
The onset, evolution and course of the illness are parameters that can lead to the etiologic diagnosis. The onset should be defined as peracute to acute (over minutes to hours), subacute (onset over days), chronic (onset over several days, weeks or months) or episodic (animal return to normal in between episodes). Inflammatory and metabolic disorders are diseases that progress over several days, while tumors and degenerative diseases have a slowly progressive course.
Current therapy which includes prescribed or nonprescription drugs is also an important factor that should be taken into consideration before the anamnesis is being finished.
Other general information that can be helpful for the diagnosis are vaccination status, diet, previous travel history, drug reactions and the animal's environment, including the potential for toxin exposure (Platt & Garosi, 2012).
The physical examination include examination of the respiratory movements, pulse, body temperature, coat and skin, mucous membrane, peripheral lymph nodes and other notable abnormalities. Carefully following these steps, the veterinarian can exclude a systemic illness that affects the nervous system, such as fever associated with meningoencephalitis, otitis in patients with vestibular syndrome (Rijnberk & van Sluijs, 2008).
The first step of the neurological examination is based on observation, as there is a lot of important information that can be gathered only this way. Using the observation, the clinician should asses the mentation, behavior, posture and the gait. Then, a hands-on neurological examination should be undertaken. That includes: postural reactions, spinal reflexes, sensory testing (superficial and deep pain),
cranial nerve examination. The order in which the different parts of the examination are completed is mainly determined by the degree of cooperation of the patient, which usually improves as the examination progresses.
Trying to perform the examination in spite of strong resistance by the patient makes both the observation and their interpretation difficult.
It is better to start the examination with the procedures that are least likely to upset the patient and delay the painful tests until the end of the examination (Adams & Brown, 2009).

Mental status and behavior

When abnormal, mentation can be a sign of forebrain or brain stem dysfunction.
Normal consciousness implies wakefulness and awareness of the environment and is asses by observing for appropriate or inappropriate response to the environment. The following levels of consciousness can be distinguished:
- alert- normal response to environmental stimuli;
- disoriented/confused- abnormal response to environment;
- depressed/obtundent- less responsive to environment;
- stuporous- unconscious but can be roused by painful stimuli;
- comatose- unconscious and unresponsive to any (including painful) stimuli. (Packer, 2013)
Common behavior abnormalities in patients with brain disease include disruptions in the pet's normal sleep-wake cycle, restlessness, aimless pacing or wandering, getting stuck in corners, the tendency to turn and cycle to one side, decreased or abnormal interaction with the family, aggression, loss of learnt behavior and excessive vocalization (Thomas, 2010).

Posture

Normal posture is characterized by symmetrical and equal bearing of weigh by all limbs, together with symmetrical positioning of the head, neck, trunk, and tail as appropriate for the breed.
Searching for postural abnormalities can be very useful as they provide important information for lesion localization. The abnormalities that can be found in the head posture are head tilt, head turn, dorsal and ventral flexion. Head tilt implies rotation of the median plane of the head, with one ear lower than the other and it indicates vestibular dysfunction (DeLahunta & Glass, 2010). With head turn or

torticollis, the head is perpendicular to the ground, but nose is turned to one side. Head turn is a sig of forebrain disease.
Decerebrate rigidity is associated with a rostral brain stem lesion and is characterized by extension of all limbs and opistotonus and implies a comatose mental status.
Decerebellate rigidity is specific for acute cerebellar lesion and is characterized by opistotonus, thoracic limbs extension, flexion of the pelvic limbs and normal mental status.
Schiff-Sherrington posture consists of extension of the thoracic limbs and paralysis of the pelvic limbs and is associated with an acute thoracic or lumbar spinal cord lesion (Platt & Olby, 2012).

Evaluation of the gait and other abnormal movements

A normal gate requires intact function of the brainstem, cerebellum, spinal cord and sensory and motor peripheral nerves, neuromuscular junctions and muscles.
Evaluation of the gait should be done with the aim of determining if the animal is ataxic, paretic or lame and which limbs are involved (Platt & Garosi, 2012).
Ataxia is an inability to perform normal, coordinated motor activity that is not caused by weakness, musculoskeletal abnormality, or abnormal movements such as tremor or myoclonus. Ataxia can evolve with hypermetria or hypometria. The 3 types of ataxia are sensory, cerebellar and vestibular.
Sensory ataxia is caused by a lesion affecting the general prioprioceptive pathways in the peripheral nerve, dorsal root, spinal cord, brain stem or forebrain. The loss of the sense of limb and body position causes clumsiness and incoordination, resulting in a wide-based stance and a swaying gait.
Cerebellar ataxia is cause by the cerebellar dysfunction and is accompanied by hypermetria and an overreaching, high stepping gait.
Vestibular ataxia is cause by a lesion of the vestibular system. Is characterized by leaning, veering, stumbling, falling, and in severe affected individuals, rolling to the affected side. The animal may stand with a broad base and exhibit exaggerated extensor tone of the contralateral limbs, accompanied by a decrease of tone in the ipsilateral limbs (Kent et al., 2010).
Paralysis is a complete loss of the motor function, whereas paresis indicates a partial loss of motor function. Based on whether a lesion affects the UMN or LMN system, two types of paresis can be distinguished: UMN paresis and LMN paresis.
UMN paresis is characterised by normal or exaggerated myotatic reflexes, and spasticity. Spasticity predominates in the antigravity muscles, and results in a stilted gait characterised by decreased limb flexion during protraction.
LMN paresis is characterised by a short-strided gait that may mimic lameness, tendency to collapse, trembling, hopping and ventral neck flexion.
Lameness is usually associated with pain from orthopaedic disease. Additionally, it can be associated with nervous system dysfunction referred to as nerve root signature (referred pain down a limb causing lameness or elevation of the limb, resulting from entrapment of the spinal nerve, usually due to a lateralized disc extrusion
or nerve root tumour). (Adams & Brown, 2009)
Abnormal movements include: tremors, myoclonus, myotonia and abnormal muscle tonus.
Tremor can be localized to one region of the body or can be generalised. A terminal tremor (intention tremor) occurs as the body parts nears a target during goal-oriented movements. Head tremor is specific for cerebellar disorders and happens when the patient tries to eat, drink or sniff an object.
A generalised postural tremor is seen in dogs with steroid-responsive tremor syndrome, intoxications or metabolic disorders.
Myotonia is a delayed relaxation of muscle following voluntary contraction. It may occur with some congenital and acquired myopathies and the percussion of a myotonic muscle often results in a dimple" appearance.
Myoclonus is a brief, shock like contraction of skeletal muscle. Encephalomyelitis caused by canine distemper is the most common cause of abnormal myoclonus in dogs. Other inflammatory diseases of the nervous system that can cause myoclonus are granulomatous meningoencephalomyelitis, bacterial encephalitis, protozoal encephalitis or steroid-responsive meningitis-arteritis.

Muscle tone may be abnormal in spinal cord injury. Muscle hypotonicity occurs when LMN lesions are present. Muscle hypertonicity or spasticity are characteristic for UMN lesions (Thomas, 2010) (Lorenz et al., 2010).

Postural reactions

Postural reactions assess the same pathways involves in gait and are useful in making a differential diagnosis between orthopaedic and neurologic disorders. The proprioceptive tests are: proprioceptive positioning, hopping, wheelbarrowing, visual placing and tactile placing, hemiwalking and hemistanding.

Proprioceptive positioning implies turning over the paw so the dorsal surface be in contact with the ground. The animal should immediately return the paw to a normal position. This test evaluates proprioception in the proximal region of the limb. A delayed or absent response indicates neurological disease.

For hopping, the animal is supported under the abdomen and allowed to weigh bear on one leg and is then moved laterally. This test is sensitive for subtle weakness or asymmetry.

Wheelbarrowing implies supporting the animal's hindquarters and walk him forwards, with the head's raised. The normal response is that the animal has to walk on the thoracic limbs without scuffing or stumbling. This test is used in order to identify any lesions that affect the thoracic limbs.

The tactile placing is tested by covering the patient's eyes and move it toward the edge of the table. When the paw touches the table, the animal should immediately place the limb forward to rest the paw on the table surface. The thoracic and the pelvic limbs are tested, and the left and right side are compared.

Visual placing is tested similarly, except that the patient's eyes are not covered. With this test, each eye can be evaluated individually by covering the contralateral eye, and the temporal and nasal visual fields can be evaluated by approaching the table from the side of the patient.

Hemiwalking can be performed by holding up the limbs on one side of the body and moving the patient laterally. Normal animals will hop on the limbs while keeping the foots under their body for support (Platt & Olby, 2012).

Spinal reflexes

Following gait and postural reactions testing, the clinician should be able to narrow down the lesion localization as being cranial to T3 spinal cord segments, caudal to T3 spinal cord segments or within the peripheral nervous system (peripheral nerve, neuromuscular junction or muscles).

Spinal reflexes evaluation helps to narrow down further the lesion localization by testing the integrity of the C6-T2 and L4-S3 intumescences, as well as the respective segmental sensory and motor nerves (LMN) that form the peripheral nerves and the muscles innervated (Platt & Garosi, 2012).

Clinically, there are a number of reflexes that can be evaluated in the animal: Extensor carpi radialis reflex, thoracic limb flexion reflex, patellar reflex, pelvic limb flexion reflex, perineal and panniculus reflex.

Myotatic reflexes are graded with regard to force of contraction, speed of contraction, and length or range of motion, using the following scale: 0-absent, 1-reduced, 2-normal, 3-exaggerated, 4-markedly exaggerated with clonus.

Weak or absent reflexes can occur with lesions that affect any part of the reflex arc, severe rigidity or muscle contraction that limits joints movement or the state of spinal shock, which can occur immediately after severe spinal cord injury.

Causes of exaggerated reflexes are lesions in the UMN pathways cranial to the spinal segment involved in the reflex or a lesion of the L6-S1 spinal segments or sciatic nerve that can cause an exaggerated patellar reflex. Exaggerated reflexes can be found in anxious or excited patients.

For the perineal reflex, a gentle stimulation is applied to the perineal area with the tip of forceps. A normal response is contraction of the anal sphincter. An absent or depressed response indicates a lesion in the sacral spinal cord or pudendal nerve.

For panniculus reflex, a pinprick stimulus is applied to the skin at the level of L5 and subsequently moved cranially. A normal response is bilateral contraction of the cutaneous trunci muscle. An absent reflex suggests a lesion slightly cranial to the point of stimulation so this test is most useful to narrow

down thoracolumbar spinal cord injuries (Dewey & da Costa, 2015).

Sensory testing

The purpose of testing pain perception is to detect and map out any areas of sensory loss. This testing aids anatomic localization and determination of prognosis.

Superficial pain sensation is tested by pinching the skin between the digits, while deep pain sensation is tested by pinching across the bone of the digit or tail.

Two types of response may be seen: a reflex flexion of the limb or skin twitch, indicating that the sensory neurons and spinal segments are intact and a behavioural response, such as crying or biting which indicates that the ascending pain pathways in the spinal cord and brain stem to the forebrain are intact.

Lack of deep pain sensation is a poor prognostic indicator of severe spinal cord injuries, because the fibres for deep pain sensation are the most resistant to injury. If deep pain sensation is lost, all spinal cord function is lost (Thomas, 2010).

Cranial nerves

There are 12 pairs of cranial nerves. Each has a different role in controlling the different functions of muscles or gland within the head. There are a series of tests that can be used to assess different combination of nerves, like menace response, pupillary light reflex, palpebral reflex, corneal reflex, response to nasal stimulation, oculovestibular reflex, gag reflex.

Cranial nerve I is tested if the owner is concerned about the animal loss of smell. The olfactory nerve is tested with a small morsel of canned food. The normal response implies a sniffing behaviour, although the patient can't see the food. The loss of smell is uncommonly recognised and is caused in general by nasal disease, rather than brain disease.

Cranial nerve II requires more than one test in order to be asses. The important tests are cotton balls dropping, menace response and pupillary light reflex. The visual following is asses by dropping cotton balls and observing if the patient's eyes and head are following the object. The menace response implies moving the hand toward the animal's eye in a threating way and observing a blink response.

The pupillary light reflex is tested with a bright light. The response should be pupillary light constriction. Blindness with normal pupils indicates a lesion of the forebrain, diencephalon, optic radiation or occipital cortex.

Cranial nerves III, IV and VI subserve eye movements and are tested together. The veterinarian should observe spontaneous eye movement when the patient looks about and then move the patient's head to induce physiological nystagmus.

Pathological nystagmus can be observed when the head is at rest or in a neutral position (spontaneous nystagmus) or when the head is moved into certain positions (positional nystagmus). Pathologic nystagmus results form a unilateral disturbance in the normal bilaterally tonic influences provided by vestibular neurons to the motor nuclei of the extraocular muscles (Rossmeisl, 2010).

The corneal sensation is tested by touching the cornea. The normal response is blink and globe retraction. Resting pupil size should be evaluated. If anisocoria is present, the doctor must determine which pupil is abnormal. Causes of an abnormally small pupil are uveitis or a painful corneal condition. Causes of an abnormally large pupil with normal vision are iris atrophy, dysautonomia or lesions of the oculomotor nerve or its nucleus in the brainstem.

Cranial nerve V provides sensory innervation to the face and motor innervation to the muscles of mastication. The 3 branches should be tested separately. The ophthalmic branch is tested by touching the medial cantus of the eye and expect for a blink response. The maxillary branch is tested touching the upper lip, lateral to the canine tooth. The normal response is wrinkling of the face and a blink. The mandibular branch is tested by touch the lower lip.

Cranial nerve VII should be asses by observing the patient's face for asymmetric eyelid closure, spontaneous blinking or a drooping ear. The facial nerve also mediated tearing, which is evaluated with Schirmer test.

Cranial nerve VIII is responsible for hearing and vestibular function. The test should include a loud noise to which the alert patient should respond orientating the head and ears toward the noise. Other signs of the nerve deficiency

include same symptoms as the vestibular syndrome.

Cranial nerves IX and X are tested together because they supply motor and sensory innervation to the pharynx. The effect of the test should be pharyngeal contraction after touching it. Also, the doctor should ask for any signs of dysphagia, voice change or inspiratory stridor.

Cranial nerve XI supplies motor innervation to the trapezius muscle and its function is difficult to be evaluated.

Cranial nerve XII innervates the muscles of the tongue. Any sign of atrophy, asymmetry or deviation of the tongue should be assessed. Also, the patient should be observed when is drinking water (Platt & Olby, 2012) (Lorenz et. al., 2010).

Intracranial lesion localization

After performing the neurological examination, all the findings have to be corelated in order to establish if the patient suffers from a neurologic disease and to localize the lesion.

For the **forebrain**, characteristic signs include alterated mental status with depression or disorientation, contralateral blindness with normal pupillary light reflex, abnormal movements like ipsilateral circling, head turn, head pressing, low postural responses in contralateral limbs. Abnormal behavior and seizures are highly predictable for forebrain lesions.

Most animals with **brainstem** lesions present abnormal mental status with depression, stupor or coma, paresis of all or contralateral limbs, several cranial nerve deficits, possibly decerebrate rigidity, accompanied by respiretory or cardiac abnormalities. Gait deficits vary from mild, ipsilateral hemiparesis to tetraplegia with normal to exaggerated spinal reflexes.

Patients with **cerebellar** lesions will present with generalized ataxia, intention tremor of head and eye and a truncal sway. A typical sign for cerebellar lesion is hypermetria. There should be no changes in mentation or behavior at all. Acute cerebellar lesions can induce decerebellate rigidity (Platt & Garosi, 2012) (Packer, 2013).

Diseases of the **vestibular system** cause varyingly severe balance and postural disturbance along with vestibular ataxia. Clinical signs may be a result of dysfunction of the peripheral or central components of the vestibular apparatus, as shown in table 1.

Peripheral vestibular disease does not affect strength or general proprioception. Spontaneous or positional horizontal or rotatory nystagmus can occur, and the fast phase will be away from the side of the lesion. Peripheral vestibular lesions can also affect the facial nerve and postganglionic sympathetic innervation to the head (Horner syndrome).

Vestibular signs associated with a depressed level of consciousness, spastic hemiparesis, cranial nerve V-XII deficits, or general proprioceptive deficits on the same side as the vestibular deficits should be considered to indicate a central vestibular disorder (Rossmeisl, 2010) (DeLahunta & Glass, 2010).

Table 1- Differentiating clinical features of peripheral and central vestibular disease (Rossmeisl, 2010)

Clinical sign	Peripheral vestibular lesion	Central vestibular lesion
Head tilt	Toward lesion	To either side
Pathologic nystagmus	Direction not altered by head position Horizontal or rotatory Fast phase away from lesion	Direction may change with head position Horizontal, rotatory or vertical
Postural reactions	Normal	Deficits ipsilateral to lesion
Conscious proprioception	Normal	Deficits ipsilateral to lesion
Cranial nerve deficits	±ipsilateral CN VII	±CNN V-XII ipsilateral to lesion
Horner syndrome	±postganglionic	±preganglionic (rare)
Consciousness	Normal Disoriented if acute	Normal to comatose

CONCLUSIONS

In order to obtain an accurate neuroanatomic diagnosis, stages of the neurological examination must be strictly followed.

All findings – normal or abnormal- should be taken into consideration for a precise localization of a lesion.

Each of the four regions of the brain is characterised by a specific series of clinical signs. The most important elements that have to be asses in order to establish a differential diagnosis are the mental status, the behaviour, the gait, the proprioception, the cranial nerves and spinal reflexes.

Revealing and interpretation of these signs is the key for a correct intracranial lesion localisation.

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MANAGEMENT AND POTANTIAL BIOGASE QUANTITIES OF WASTE FROM ANIMAL BREEDING ENTERPRISES: ANTALYA CASE

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Abstract

In the livestock enterprises, the wastes which are produced after the production can be shown as a pollutant source when not properly managed. Wastes from livestock enterprises must be stored and managed properly. The use of renewable energy sources is increasing due to the reduction of fossil energy resources and the environmental pollution of these resources. Biogas energy production among renewable energy sources in the direction of developing technology and increasing population needs can be applied in areas where animal breeding facilities are carried out. In case of animal wastes reaching underground and surface water resources for biogas production, water pollution will occur. Therefore, the properly storage of wastes on animal barns and transfer to biogas plants may remove the potential negative effects on water resources. In cases where waste from livestock enterprises is not stored properly, these enterprises will be the source of pollutants for water resources. In study area, primarily livestock potential enterprises that will cause pollution problems to water resources have been determined. Potential biogas quantities to be generated from these facilities have been calculated and the potential contributions of livestock enterprises to energy costs have been investigated.

Key words: *Antalya, Animal waste, Biogas, Water pollution.*

INTRODUCTION

Wastes that develop as a result of industrial and agricultural activities are among the primary reasons of water pollution (Kaplan et.al., 1999). In this regard, nitrogen and phosphorous are especially important with regard to both underground water pollution and eutrophication in surface waters. Eutrophication along with related ecological impacts prohibit the use of all water resources as drinking and service water in addition to endangering them (Sharpley, 1995; Anonymous, 2000).

Animal wastes may pollute surface and underground water resources as a result of uncontrolled waste management. This is caused due to impacts related with the animals directly reaching a water source, manure piles, surface flow waters seeping from shelters and open feeding areas, seepage from manure storage structures, submergence of storage areas in addition to surface flow from manure application areas and pastures (Anonymous, 2005).

Pollution of waters with nitrate (NO_3) is among the primary fields of interest among manure related pollution. Because nitrate (NO_3) is increasingly accumulated in the soil with manure that is defined as output in animal production and as input in plant growth. Certain amounts of this accumulated nitrate depending on various conditions such as soil, topography, climate etc. are washed and reach various depths of the soil and mostly the surface and underground water sources (Kaplan et. al. 1999).Nitrate pollution in underground waters attracts worldwide attention as a significant problem for areas that are near agricultural fields (McLay et.al., 2001).

A significant amount of manure is obtained in livestock businesses depending on capacity. Manure processing is a primary concern for many businesses and issues such as the cleaning, removal, storage and use of manure are either not noticed or emphasized. Solid and liquid manure that is obtained at various plants is either removed in an uncontrolled manner or is thrown away. The failure to make use of organic manure results in wasting of national wealth in addition to significant environmental issues (Şimşek et.al., 2001).

Biogas that is produced by transforming organic wastes into methane gas in an oxygen free environment can be used as a source of heat and electricity in businesses and the materials that remains at the end of the process can also be used as an enriched source of manure (Gül, 2006). The sulfur emission of biogas fuels is almost zero, they reduce acid rain, contribute to the atmospheric carbon cycle while preventing the increase of global warming (Saraçoğlu, 2004).

The study was carried out at the Antalya district of the Mediterranean Region in Turkey where people are mostly engaged in agriculture. Greenhouse cultivation is popular at the coastal parts of the city due to suitable climate conditions, however the number of livestock businesses increases with increasing distance from the sea. The objective of the study was to evaluate the total numbers of cattle per district in addition to the potential pollution and potential biogas production values for the total number of cattle. It has been considered that the study results shall contribute to the betterment of manure utilization applications in the study region as well as raising awareness regarding the pollution potential for water sources.

MATERIAL AND METHODS

Data regarding the capacities of cattle breeding businesses active in the region were acquired from 2016 VETBIS system data by way of Antalya Directorate of Provincial Food, Agriculture and Livestock. Livestock businesses were determined based on a consideration of their potential to pollute the water resources. In this regard, livestock businesses at the districts of Alanya, Manavgat, Korkuteli and Elmalı comprise the study material. Whereas the potential biogas energy amounts that can be obtained from businesses were calculated according to Hill (1982) and Ekinci et.al. (2010). Average amount of manure obtained from adult cattle can be taken as 43 kg/day for the calculation of manure amount. Accordingly, an adult cattle produces 1-1.5 tons manure/month. The energy value of the biogas volume due to cattle manure can be considered 20 MJ m^{-3} (Ekinci et.al., 2010). The MJ value that is taken as basis corresponds to the thermal energy value for the annual amount of manure that can be acquired from one cattle. In addition, the distance to water sources of the villages where the cattle breeding businesses in the study area are located in along with the elevation differences between the business and water source have been determined via Google Maps (Anonymous, 2018).

RESULTS AND DISCUSSION

Greenhouse cultivation in the city of Antalya is carried out in areas close to the coast with plant production as the primary agricultural output. The fact that the Taurus Mountains are located to the north of the city decreases the microclimate effect and thereby ovine and cattle breeding or fruit gardens resistant to cold climate are observed more in these areas. It is observed that the wastes and manure from cattle businesses in the region are directly discharged to the rivers. It was determined as a result of a comparison between the districts in the study region that livestock breeding is carried out in villages near water sources especially in the Korkuteli and Elmalı districts. When the total cattle numbers of the district in the study area were examined, it was determined that the cattle capacity of Korkuteli, Alanya, Elmalı and Manavgat are 20214, 4952, 10615 and 5734 respectively and that the distance of the businesses to the water sources was less than 5 km. Potential wet manure and biogas energy amount was calculated based on the number of animals in the study region according to Hill (1982) and Ekinci et.al. (2010). The distances to the water sources and potential pollution levels were tried to be determined for the wet manure amount from the businesses in the study region.

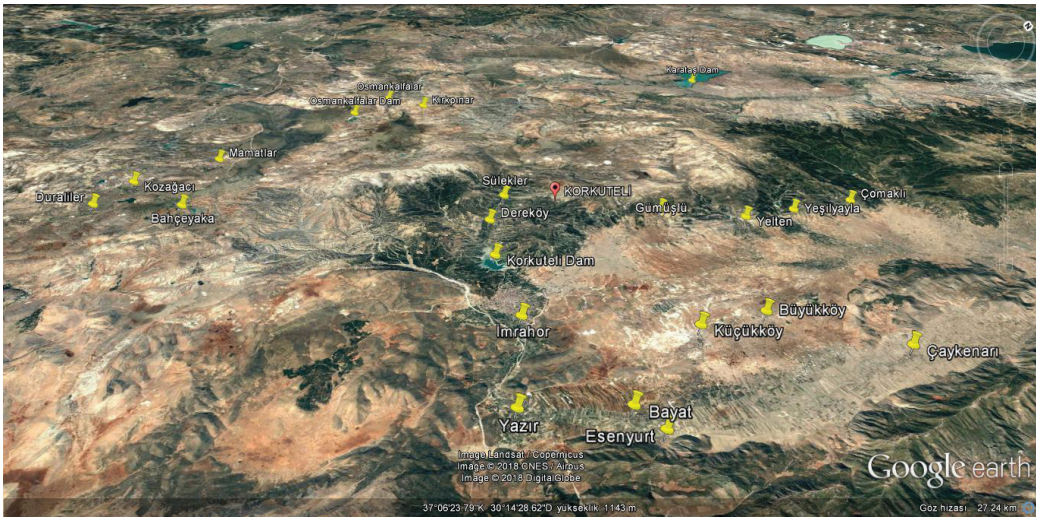

Figure 1. Satellite views of cattle breeding enterprises that may create potential pollution in the Korkuteli District.

It was observed that the businesses in the region do not aim to store their manure and wastes, that they do not give importance to storage structures and that they are not very sensitive towards the environment.

It is specified in studies carried out in our country that the livestock breeding businesses do not give necessary importance to storing the manure formed (Saltuk, 2017).

Therefore, similar findings were observed in the study area. Especially businesses in rural regions away from the district centers spread out the manure on the field and let it wait there. Wet manure amount and potential biogas energy amount that can be obtained from the Korkuteli district with the highest number of cattle in the study region have been given in Table 1.

Table 1. The amount of potential wet manure and obtainable biogas energy in the Korkuteli District

Village	Animal numbers	Water source	Distance of water source (km)	Altitude difference (m)	Annual average amount of wet manure (kg)	Annual amount of obtainable biogas energy (MJ)
Bahçeyaka	385	Kızıldere	0.6	49	6042575	2528716,485
Bayat	2972	Korkuteli River	0.5	2	46645540	19520377,64
Büyükköy	2262	Korkuteli River	5	26	35502090	14857030,36
Çaykenarı	601	Irrigation Canal	1.2	3	9432695	3947424,954
Çomaklı	1890	Dam	5	6	29663550	12413699,11
Dereköy	319	Korkuteli River	0.5	27	5006705	2095222,23
Duraliler	131	Kozağacı Pond	1.8	105	2056045	860420,4143
Esenyurt	150	Korkuteli River	0.4	10	2354250	985214,2149
Gümüşlü	830	Yelten Pond	5	192	13026850	5451518,656
İmrahor	152	Korkuteli River	0.5	12	2385640	998350,4044
Kırkpınar	636	Osmankalfalar Pond	3	184	9982020	4177308,271
Kozağacı	450	Kozağacı Pond	1	20	7062750	2955642,645
Küçükköy	2505	River	1.7	83	39315975	16453077,39
Mamatlar	533	Irrigation Canal	1.5	38	8365435	3500764,51
Osmankalfalar	300	Osmankalfalar Pond	2	57	4708500	1970428,43
Sülekler	368	Korkuteli River	0.8	2	5775760	2417058,874
Yazır	763	Korkuteli River	1	6	11975285	5011456,306
Yelten	3383	Yeşilyayla Pond	3.8	20	53096185	22219864,59
Yeşilyayla	1584	Yeşilyayla Pond	1.2	14	24860880	10403862,11
Toplam	20214	-	-	-	317258730	132767437,6

As can be seen Table 1, Yelten village has the highest wet manure and biogas energy amount that can be potentially obtained in the Korkuteli district.

Bayat village with the second highest cattle number in the district has the highest probability of polluting the water sources with animal wastes.

It was determined that the distance to the water sources in 6 out of the 19 villages with cattle in the district is less than 1 km. The businesses in these villages close to the water sources made up 21.5% of the total district potential. It was determined upon an evaluation of proximity to water sources and the number of animals that 6 villages of the Korkuteli district were about 0 - 0,8 km away from water sources, whereas the distances of 9 villages from water sources was 1-2 km and that the remaining 4 villages were 2,1 - 5 km away from water sources.

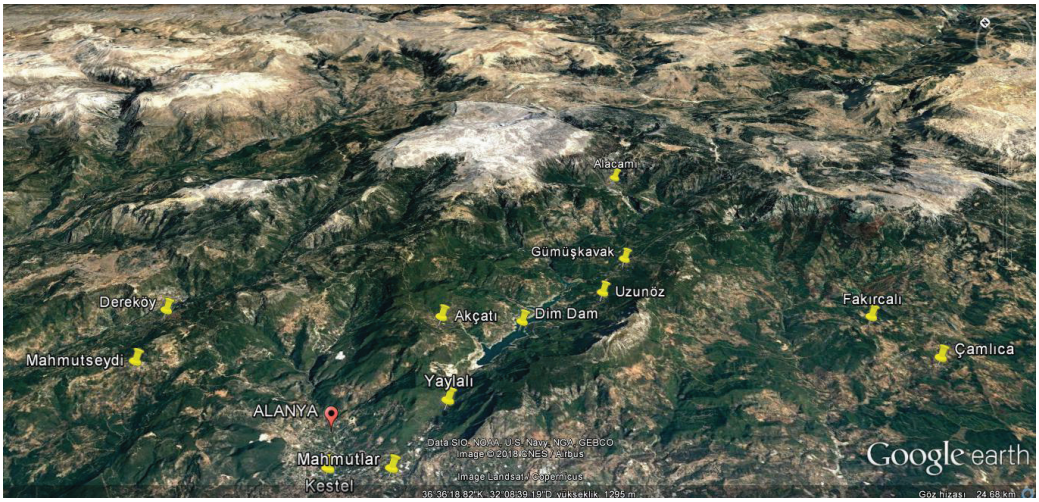

Figure 2. Satellite views of cattle breeding enterprises that may create potential pollution in the Alanya District

Wet manure amount and potential biogas energy amount that can be obtained from the Alanya district in the study region have been given in Table 2.

Table 2. The amount of potential wet manure and obtainable biogas energy in the Alanya District

Village	Animal numbers	Water source	Distance of water source (km)	Altitude difference (m)	Annual average amount of wet manure (kg)	Annual amount of obtainable biogas energy (MJ)
Akçatı	88	Dim Dam	2	602	1381160	577992,340
Alacami	321	Dim River	4	262	5038095	2108358,420
Çamlıca	692	Oba River	5	270	10860940	4545121,578
Dereköy	906	Dim River	1	359	14219670	5950693,858
Fakırcalı	118	Oba River	1.8	471	1852010	775035,182
Gümüşkavak	260	Dim Dam	2	343	4080700	1707704,639
Kestel	538	Dim River	2	20	8443910	3533634,984
Mahmutlar	998	Dim River	5	15	15663610	6554958,576
Mahmutseydi	606	Dim River	3.2	264	9511170	3980265,428
Uzunöz	172	Dim Dam	2	493	2699540	1129712,300
Yaylalı	253	Dim Dam	1	42	3970835	1661727,975
Toplam	4952	-	-	-	77721640	32525205,280

It was observed that the number of cattle in the Alanya district was low in comparison with the province in general, but that the number of animals increased especially in businesses that are close to the water sources. It was concluded that solid and liquid wastes of

businesses that are close to the water sources may mix in with the surface waters thus resulting in environmental pollution as well as economic losses. As can be seen in Table 2, Mahmutlar village has the highest wet manure and biogas energy amount that can be obtained from the district of Alanya. It is observed that Yaylalı village has the highest probability of polluting the water sources with wastes from cattle.

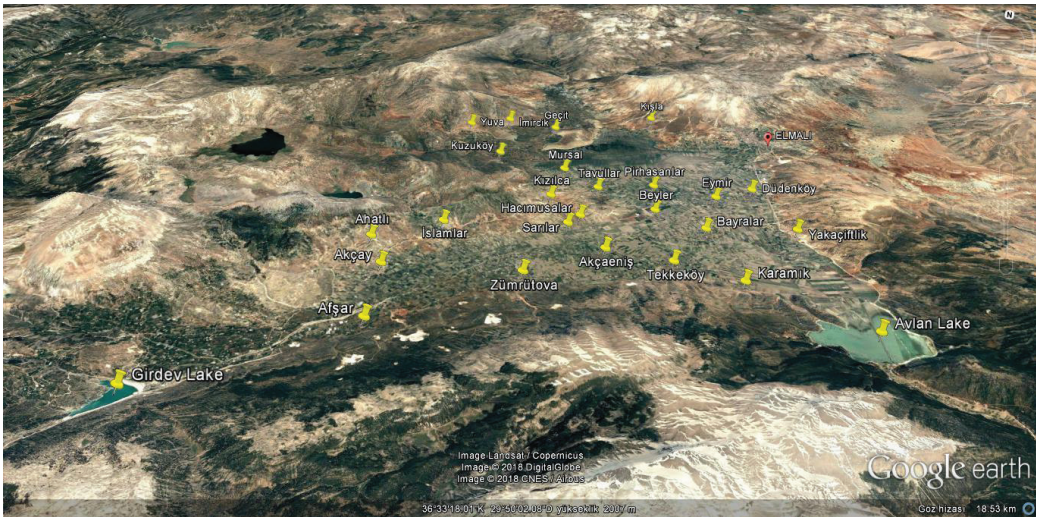

Figure 3. Satellite views of cattle breeding enterprises that may create potential pollution in the Elmalı District

The amount of wet manure and potential biogas energy amount that can be obtained from the district of Elmalı have been given in Table 3.

Table 3. The amount of potential wet manure and obtainable biogas energy in the Elmalı District

Village	Animal numbers	Water source	Distance of water source (km)	Altitude difference (m)	Annual average amount of wet manure (kg)	Annual amount of obtainable biogas energy (MJ)
Afşar	122	Akçay	0.5	4	1914790	801307,561
Ahatlı	72	Gavurçay River	2.8	59	1130040	472902,823
Akçaeniş	181	Akçay	0.9	2	2887880	1188825,153
Akçay	155	Akçay	2.1	18	2432725	1018054,689
Bayralar	1172	Akçay	0.5	2	18394540	7697807,065
Beyler	54	Akçay	2	1	847530	354677,117
Çaybaşı	197	Akçay	0.5	2	3091915	1293914,67
Düdenköy	377	Kurutma Canal	0.5	3	5917015	2476171,727
Eymir	388	Kurutma Canal	2.5	3	6089660	2548420,769
Geçit	381	Kurutma Canal	0.7	2	5979795	2502444,105
Hacımusalar	155	Akçay	1.4	7	2432725	1018054,689
İmircik	94	Kurutma Canal	2	13	1475330	617400,907
İslamlar	55	Gavurçay River	0.4	7	863225	361245,212
Karamık	92	Avlan Lake	3.4	17	1443940	604264,718
Kışla	908	Kurutma Canal	3.5	15	14251060	5963830,047
Kızılca	108	Akçay	3.4	72	1695060	709354,235
Kuzuköy	124	Kurutma Canal	4.3	11	1946180	814443,750
Mursal	354	Kurutma Canal	3.8	13	5556030	2325105,547
Pirhasanlar	112	Kurutma Canal	5	2	1757840	735626,613
Sarılar	164	Akçay	1	6	2573980	1077167,541
Tavullar	624	Akçay	4	1	9793680	4098491,134
Tekkeköy	289	Akçay	2.2	4	4535855	1898179,387

Yakaçiftlik	601	Kurutma Canal	0.2	13	9432695	3947424,954
Yuva	3706	Kurutma Canal	3	18	58165670	24341359,201
Zümrütova	130	Akçay	1.7	10	2040350	853852,320
Toplam	10615	-	-	-	166602425	69720325,938

The fact that businesses in the Elmalı district are small and family owned may decrease potential pollution and the fruit gardens in the region such as apple, peach and plum allows for the use of the generated manure. However, it should be kept in mind that the generated manure should be stored in a suitable environment for this purpose. The nutrients in the manure can be preserved in this manner while also eliminating the pollution problem. Table 3 indicates the highest amount of wet manure and biogas energy in the Elmalı district can be obtained from the Yuva village. While Bayralar village with the second highest number of cattle in the district was determined to have the highest probability of polluting the water sources.

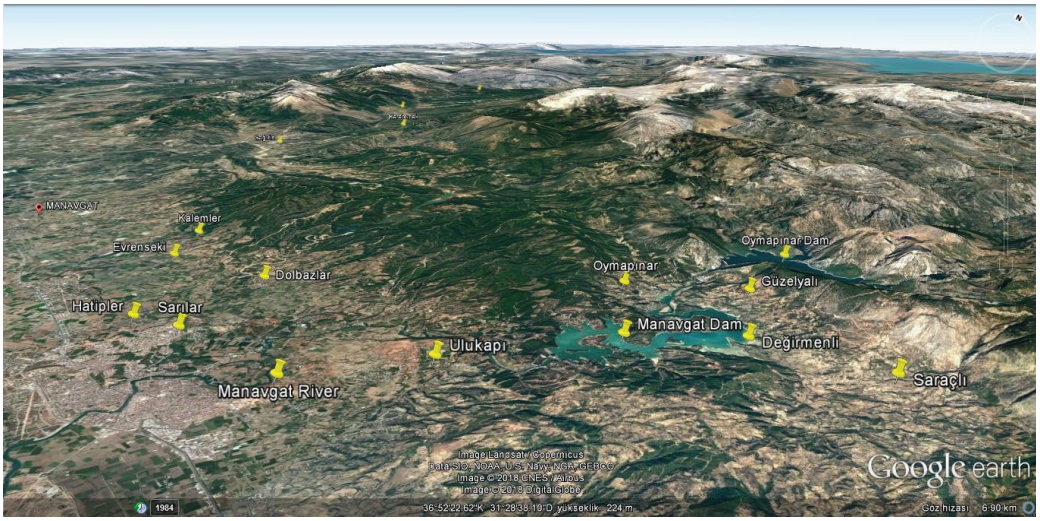

Figure 4. Satellite views of cattle breeding enterprises that may create potential pollution in the Manavgat District

Amounts of wet manure and potential biogas that can be obtained from the Manavgat district have been given in Table 4.

Table 4. The amount of potential wet manure and obtainable biogas energy in the Manavgat District

Village	Animal numbers	Water source	Distance of water source (km)	Altitude difference (m)	Annual average amount of wet manure (kg)	Annual amount of obtainable biogas energy (MJ)
Değirmenli	114	Manavgat Dam	0.2	12	1789230	748762,803
Dolbazlar	132	Manavgat River	4.2	22	2071740	866988,509
Evrenseki	1008	Kargıçayırı River	4.6	17	15820560	6620639,523
Gaziler	72	Köprüçay	1.5	55	1130040	472902,823
Güzelyalı	141	Manavgat Dam	1	94	2212995	926101,362
Hatipler	77	Manavgat River	3.4	39	1208515	505743,297
Oymapınar	51	Manavgat Dam	0.5	23	800445	334972,833
Kalemler	447	Kargıçayırı River	3.5	50	7015665	2935938,360
Karabucak	482	Köprüçay	4	276	7564990	3165821,677
Karabük	199	Köprüçay	0.4	38	3123305	1307050,858
Ulukapı	1449	Manavgat River	2.8	71	22742055	9517169,315

Sağırin	1092	Köprüçay	2	20	17138940	7172359,484
Saraçlı	318	Manavgat Dam	4	84	4991010	2088654,135
Sarılar	152	Manavgat River	2	46	2385640	998350,404
Total	5734	-	-	-	89995130	37661455,38

As can be seen in Table 4, the highest wet manure and biogas energy amounts in the Manavgat district can be obtained from the Ulukapı village. Whereas Değirmenli village has the highest probability of polluting water sources with cattle waste. Nitrate level in the well waters near the manure storage areas of businesses may reach dangerous levels for humans and animals. Excessive nitrate intake may result in pain, vomiting as well as comas and deaths in animals (Kaya and Akar, 2002). It may lead to the onset of various hereditary diseases in humans as a result of the impairment of the immune system (Weyer et al., 2001). Manure and wastes that are generated as a result of any livestock breeding activity should be stored in suitable environments. In addition, the animal manure generated can be transformed into biogas and electrical energy thus decreasing the energy costs of businesses while also paving the way for a greener environment (Bilgin, 2003).

CONCLUSION

A total of 651577925 tons of wet manure may potentially be obtained from the cattle in the study region. In addition, it has also been determined that a total of 272674424,2 MJ biogas energy amount can be obtained from this wet manure. Energy costs of businesses may be decreased if this potential biogas energy can be used in the businesses. It can be stated that the potential pollution due to cattle breeding businesses in the region may be high in the Elmalı and Korkuteli districts depending on the number of cattle and that the districts of Alanya and Manavgat may have lower levels of potential pollution. Amount of manure generated at the businesses vary depending on the number of cattle. It should be kept in mind that the generated manure has different ratios of organisms that are harmful for health and thus their contact with water sources should be prevented. It has been observed that no precautions have been taken in majority of the cattle breeding businesses in the study area for the storage of the liquid or solid wastes without harming the environment. Animal wastes that are randomly stored on lands that are close to the water sources may mix in with the surface waters, accumulate around the living areas thus resulting in the formation of odors and flies. The demands of the region for potential new and renewable energy sources may be met from these areas and the proper storage of manure may contribute to plant production while also reducing the energy costs of the businesses by way of the potential biogas and electrical energy it may supply.

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INFLUENCE OF PROBIOTICS CLOSTAT® AND LAKTINA® ON THE AMINO ACID COMPOSITION OF PHEASANT MEAT

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Abstract

The main objective of our study was to determine the impact of CloSTAT® and Laktina® probiotics on the amino acid composition of pheasant meat. The experiment included 90 one-day pheasant chicks (Phasianus colchicus colchicus), divided into 3 groups grown under free access to food and water for 3 months. All birds were fed with standard pheasant feed, to the second group it was added CloSTAT® probiotics (0.5 g / kg fodder); and Laktina® probiotic (0.5 g / l water) was added to the third group.
At the end of the experiment, five pheasants were sampled from each group after slaughtering,. The following amino acids have been tested: asparagine, threonine, serine, glutamine, proline, cystine, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine. The results of the experiment show that the probiotic CloSTAT® influences, albeit not statistically-significantly, the amino acid composition of pheasant meat.

Key words*: Pheasants, meat, amino acids, probiotics.*

INTRODUCTION

Probiotics are real alternative to the nutrition antibiotics. They are defined as viable microorganisms (bacteria or yeast) that competitively exclude colonization of intestinal pathogens and demonstrate a beneficial effect on the health of the host when ingested (Salminen et al., 1998). Probiotics are oldest feed additives in poultry nutrition (Gálik, 2012). Kabir (2009), Ivanović et al. (2012) and Maiorano et al. (2012) studied the impact of probiotics on meat quality in broilers, but nobody explored the impact of probiotics on pheasant's meat.
As reported by Tucak et al. (2004) biological value of the meat of pheasants which were fed naturally is higher in comparison to the meat of pheasants fed with commercial mixtures.
In many countries, the pheasant is selected with the aim of producing high quality meat with very desirable nutritional values (Santos Schmidt *et al*. 2007). There are only few publications on amino acid composition of pheasant meat. The knowledge of amino acid composition of pheasant meat can be used to determine its potential nutritional value. Pheasant meat is consumed relatively rarely in comparison with hen meat, pork, or beef (Chisholm *et al*. 2008). Straková *et al*. (2006) compared the amino acid composition of pheasant and chicken meats at the age of 42 days. The knowledge of the amino acid composition of food is very important. It is useful for the determination of the potential nutritional value (Young & Pellett 1984). The main objective of our study was to determine the impact of CloSTAT® and Laktina® probiotics on the amino acid composition of pheasant meat.

MATERIALS AND METHODS

The experiments were carried out with 90 one-day pheasant chicks (*Phasianus colchicus colchicus*) divided into 3 groups of 30 birds each (I group – control; II group – experimental with probiotic CloSTAT®; III group - experimental with probiotic Laktina®). They are bred on the floor, in controlled environment, with an extended light period (24 h / day) and free access to food and water for 90 days. All pheasants were received identical in composi-

tional and nutritional value of standard commercial feed mixtures for pheasants, balanced by protein, energy, amino acids, etc., according to the requirements of NRC (1994). Nutritional value of the feed mixture are presented in Table 1.

Table 1.Chemical composition of feeding mixtures

Nutritive value	Starter (0-28 day)	Grower (29-90day)
Moisture,%	11,1	11,8
ME, (Kcal/kg)	2872	2912
ME (MJ/kg)	12	12,2
Crude Protein,%	28	24,1
Crude Fats,%	3,6	3,3
Linoleic acid,%	1,6	1,4
Crude Fiber,%	3,8	3,6
Crude ash,%	5,8	5,5
Ca,%	1,07	0,98
Available phosphorus,%	0,54	0,51
Phosphorus,%	0,84	0,8
Sodium,%	0,21	0,18
Chlorine,%	0,21	0,22
Chlorides,%	0,3	0,33
Lysine,%	1,7	1,41
Methionine,%	0,54	0,5
Methionine + Cysteine,%	1	0,93
Treonine,%	1,05	0,92
Tryptophane,%	0,35	0,3
Arginine,%	1,85	---

From hatching up to 28 days, the pheasants were fed with a "Starter" commercial feed mixtures with 28% crude protein and ME 2872 Kcal / kg, and from 29 to 90 days with "Grower" commercial feed mixtures with 24.1% crude protein and ME 2912 Kcal / kg.

To the commercial feed mixtures for the first group (I group - positive control) for prophylaxis was added semduramicin sodium as a commercial product Aviax 5% (Phibro Animal Health Corporation) and to the drinking water was added antibiotic growth promoter Enrofloxacin and Colistin as a commercial product QUINOCOL (CEVA SANTE ANIMALE, France) in dose (1 ml / 2 l of water) from the 1st to the 5th day. To the commercial feed mixtures for the second group (II group) was added probiotic CloSTAT® (Kemin, Inc., USA) in dose (0,5 g / kg forage); and nothing to the drinking water was added. To the commercial feed mixtures for the third group (III group) nothing was added and to the drinking water was added probiotic Laktina® (Lactina, Bulgaria) in dose (0,5 g / l of water). All doses used in this study are by the recommendation of the manufacturer.

All birds was vaccinated as follow: against Newcastle with vaccine CEVAC BI L® by instillation into the eye according to the instructions for use of the vaccine from the manufacturer, at the 7th, 28th, 64th and 80th day; against Gumboro with vaccine CEVAC GUMBO L® by drinking water according to the instructions for use of the vaccine, at the 14th and the 22nd day; and against Avian Pox with vaccine CEVAC FP L® by applying in the wing fold according to the instructions for use of the vaccine-on the 56th day.

In this study was used suplements like: probiotic CloSTAT® (Kemin, Inc.) containing: *Bacillus subtilis* $2x10^7$ cfu / g spores, Maltodextrine, Calcium Carbonate and probiotic Laktina® (Lactinia Ltd.) containing *Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus casei, Bifidobacterium longum, Lactobacillus acidophilus* tbc in 1g not less than 1 billion. Laboratory analysis to establish the quality of the fresh meat of pheasants were performed separately for breast and leg muscles in all three experimental groups.

Samples were taken from the pectoral muscles (breast) and femoral muscles (leg).

The muscle was separated from the bones and the skin and subcutaneous fat were also removed. The determination of the amino acid composition of the pheasant meat was made using an automated amino acid analyzer based on the principle of ion-exchange column chromatography by the method of Moore and Stein (Moore and Stein, 1963). In this study, the following amino acids were deteminated: asparagine, threonine, serine, glutamine, proline, cystine, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine. All data in our study were analysed statistically using the Program StatMost 3.6, Dataxiom Software, 2003. The results are expressed as means ± SD (standard deviation). The level of statistical significance was at $P \leq 0.05$.

RESULTS AND DISCUSSION

The data of our analysis of amino acid content of breast and leg muscles of pheasants are presented in Table 2.

Table 2. Comparative amino acid composition of the pheasant meat in breast and led muscles(n = 5)

Amino acids		breast muscles			leg muscles		
g/100 g fresh meat		I group control	II group Clostat	III group Lactina	Igroup control	II group Clostat	III group Lactina
asparagine	%	2,17 ±0,16	2,17 ±0,12	2,07 ±0,08	2,31 ±0,14	2,64 ±0,11	2,40 ±0,13
threonine	%	0,834 ±0,04	0,864 ±0,05	0,819 ±0,05	0,927 ±0,03	1,060 ±0,05	0,991 ±0,04
serine	%	0,62 ±0,08	0,66 ±0,09	0,62 ±0,06	0,65 ±0,06	0,74 ±0,05	0,78 ±0,07
glutamine	%	3,98 ±0,02	4,10 ±0,11	3,99 ±0,11	4,24 ±0,11	4,85 ±0,10	4,17 ±0,13
proline	%	0,98 ±0,03	1,06 ±0,04	1,01 ±0,03	0,10 ±0,04	1,13 ±0,05	1,03 ±0,06
cystine	%	0,23 ±0,04	0,26 ±0,08	0,27 ±0,02	0,31 ±0,02	0,35 ±0,09	0,27 ±0,06
glycine	%	1,04 ±0,06	1,07 ±0,03	1,04 ±0,06	1,03 ±0,08	1,18 ±0,08	1,05 ±0,07
alanine	%	1,26 ±0,02	1,30 ±0,03	1,29 ±0,02	1,43 ±0,02	1,63 ±0,02	1,38 ±0,03
valine	%	1,12 ±0,01	1,13 ±0,03	1,13 ±0,01	1,40 ±0,01	1,60 ±0,03	1,35 ±0,02
methionine	%	0,25 ±0,16	0,29 ±0,14	0,37 ±0,04	0,525 ±0,10	0,600 ±0,22	0,344 ±0,14
isoleucine	%	1,07 ±0,01	1,01 ±0,02	1,05 ±0,03	1,26 ±0,06	1,44 ±0,04	1,23 ±0,03
leucine	%	1,80 ±0,02	1,83 ±0,03	1,79 ±0,05	2,07 ±0,02	2,37 ±0,03	2,04 ±0,04
tyrosine	%	0,65 ±0,08	0,66 ±0,13	0,68 ±0,02	0,79 ±0,10	0,90 ±0,10	0,77 ±0,09
phenylalanine	%	0,89 ±0,02	0,91 ±0,02	0,89 ±0,01	0,98 ±0,01	1,12 ±0,02	0,95 ±0,01
histidine	%	0,83 ±0,02	0,87 ±0,03	0,82 ±0,03	1,70 ±0,03	1,94 ±0,02	1,72 ±0,03
lysine	%	2,03 ±0,02	2,08 ±0,05	2,05 ±0,08	2,32 ±0,02	2,66 ±0,05	2,33 ±0,06
arginine	%	1,25 ±0,04	1,30 ±0,03	1,21 ±0,01	1,50 ±0,03	1,71 ±0,04	1,55 ±0,08
Total	**%**	**20,95**	**21,64**	**21,24**	**24,41**	**27,91**	**24,34**

The results of the amino acid profile of the breasts and legs meat showed, that nine of ten possible essential amino acids have been identified. There is no tryptophan, which is an essential amino acid and generally in pheasant meat has the lowest values - 0.29 % of all essential amino acid by scientific data (Petkov, R., 1999). One of other amino acids (essential and semi-essential) hydroxyproline was not found, which according to the literature also has the lowest values – 0.04 % (Petkov, R., 1999). The remaining essential amino acids are within the permissible limits for pheasant meat, which confirms the biological value of the meat. The study conducted by Brudnicki et al. (2012) reported that the meat of the farm pheasants in comparison to that of the wild pheasants was characterized by higher levels of 12 from the total of 15 amino acids analysed.

Comparing the results of the amino acid composition of pheasant meat with the amino acid profile of poultry meat, an increase in the essential amino acids in pheasants except tryptophan was found, which is not found in our studies. The resulting amino acid profile for breasts meat and leg meat showed of a high biological value of the protein in the pheasant meat of the experimental groups. The total amino acid content in the meat of pheasants receiving the probiotic CloSTAT® was higher than that of the control group, and the group received the probiotic Laktina®. Similar is the trend for both types of meat, more pronounced for leg meat than for breast meat.

CONCLUSIONS

Nine of ten possible essential amino acids have been identified in the meat of pheasants.

The total amino acid content in the meat of pheasants receiving the probiotic CloSTAT® was higher than that of the control group. Hydroxyproline and tryptophan was not found in the pheasant meat from all tested groups. The results of the experiment show that the probiotic CloSTAT® influences, albeit not statistically-significantly, the amino acid composition of pheasant meat.

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IN VITRO TRIAL ON USING AMPROLIUM CLORHIDRAT TO CONTROL *NOSEMA* INFECTION IN HONEY BEES

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Abstract

Nosema spp., a microsporidian parasite (Microspora: Microsporidida), is well known for the negative impact on the bee colony. In areas with temperate climate, the nosemosis's evolution in the apiary is different from season to season. During spring, when in the hive, due to the consumption of honey and bee bread reserves contaminated with spores, the infectious pressure is increasing, the disease worsens, and there is need for a medical intervention. For this, there is an increasing interest for additional products to control this infection. Therefore, this study aimed to test amprolium hydrochloride 20% ($C_{14}H_{19}CIN_4$), a product which has a structure similar to the B1 vitamin (thiamine) towards which it is a competitive antagonist, for controlling Nosema infection in honey bees. The trial was carried out under laboratory conditions and the microclimate parameters have been monitorized. There were used two experimental modules and one control, each module consisting of two batches of bees naturally infected with Nosema spp., with at least 100 bees (122-185) per batch. The batches have been organized according to the current standards, in wooden cages (with the size of 190/150/50 mm) equipped with window, ventilation mesh and feeder. Throughout the trial, the product has proved to control the development of the parasite so as at an initial infection level of approx. 5,750,000 spores/bee, by the end, in the experimental batches no spores of Nosema spp. were detected. Also, there were not signs for adverse effects on bees. However, additional field and laboratory studies are necessary toward to develop an integrated control program over the bees' active season.

Key words*: Nosema; honeybee; amprolium; in vitro trial.*

INTRODUCTION

Microsporidida includes obligatory intracellular parasites forming single-cell spores that infect different hosts; of them, *Nosema* species commonly infect invertebrates, including Hymenopteran insects.

Two species of *Nosema* genus, described as *Nosema apis* and *Nosema ceranae*, cause a serious diseases in honey bees called nosemosis, well-known all over the world for its negative impact on honey bees. Spores of *Nosema* spp. are unicellular and have an extrusive polar tube, long and spiral filament, for the host penetration. Vegetative forms develop in the host, strictly intracellular, in the epithelial cells of the middle intestine, causing digestive dysfunction and metabolic disorders (Aioanei et al., 2011; Mitrea, 2011).

Nosemosis' occurrence in the bee colony, especially in temperate areas shows differences from season to season. This disease is considered to be a major problem during spring, when an imbalance in the host-parasite relationship occurs, with a negative effect on the survival and / or on the production capacity of the honeybee colony (Bailey, 1976; Chioveanu, 2009; Crane, 1975).

Due to the ubiquity of the spores and the precariousness of the host-parasite relationship equilibrium, it is necessary to constantly monitor the disease and to intervene quickly during of critical periods, such as: in spring after winter, in spring and autumn during of

colony unifications and during of the active season, subsequently of the formation of artificial swarms (Chioveanu et al., 2004; Glavinic et al., 2017; Popovici et al., 2012).

At the end of the pause period, in spring, when inside the hive, due to the consumption of honey and beebread contaminated with spores, the infectious pressure increases, the disease becomes acute and treatment is needed. In critical periods, it is necessary to support the bee colony with energy and / or proteic bio-stimulators, supplemented with suppressing drug products (fungi-static or fungicidal ones) against the parasitic stages. In this respect, there is an increasing interest on finding additional products with action against of the *Nosema* microsporidia parasites (Chioveanu et al., 2004; Glavinic et al., 2017)

Given to the anticoccidian spectrum on different species (such as fish, birds, mammals) and starting from the efficiency of Amprolium hydrochloride treatment (icbmv.ro), the aim of the present study was to test under laboratory conditions the potential and efficacy of this product to control *Nosema* infection in honey bees.

MATERIALS AND METHODS

In this study, the product Amprolium hydrochloride 20% solution ($C_{14}H_{19}CIN_4$) – a veterinary antiprotozoal (coccidiostat) that interferes with thiamine metabolism, was assessed. The product has a similar structure to vitamin B1 (thiamine), required by the enzymatic system of glucose metabolism, to which it is a competitive antagonist (http://www.icbmv.ro/).

The biological material used in the study was represented by honey bees (*Apis mellifera*) from an apiary in Prahova county (Valenii de Munte, Romania). Before organizing the experimental trial, a pre-selection study was performed to determine the level of *Nosema* infection by investigating 7 colonies for selection of those colonies appropriate for the experimental study (Table 1).

The level of infection with *Nosema* spores was determined in laboratory using a quantitative method. Briefly described: for each analyzed sample, 60 bee abdomens were macerated in 60 ml of distilled water (ddH_2O). The resulting suspension was centrifuged for 6 minutes at 800 r/min. The sediment was re-suspended, supplementing it to 60 ml with distilled water and examined for presence of *Nosema* spp. spore. From each sample, 10 µl were used to load a Burker-Turk hemocytometer counting chamber and spores were counted (5 aliquots/sample were counted and based on the results the arithmetic mean was determined, obtaining the number of spores/an analyzed unit) (Chioveanu et al., 2009; Ionita and Mitrea, 2013; OIE, 2013).

Table 1. Data on the level of *Nosema* infection (no. of spores) in the investigated bee colonies for selection of the bee batches

Level of *Nosema* infection in the investigated bee colonies (colony ID)	**Number of positive colonies**	**Selection as**
Colonies with 23 spores/unit = 5 750 000 spores/ bee (ID: 724)	1	Experimental colony
Colonies with 1 spore/unit = 250 000 spores/ bee (ID: 759; 778; 625;793)	4	Clinical healthy colonies
Colonies with 5 spores/unit = 1 250 000 spores/bee (ID: 721)	1	Reserve colony
Colonies with 8 spores/ unit = 2 000 000 spores /bee ID: 767)	1	Reserve colony

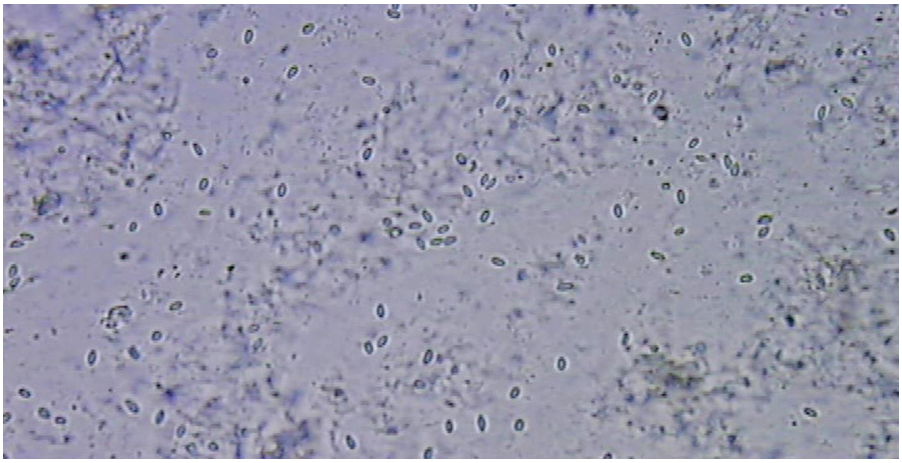

Figure 1. *Nosema* spp. spores in honey bees (direct smear; x 400)

In order to perform „in vitro" trial, two experimental modules were selected from the colony ID-724 where it has been found an infection with *Nosema* spp. of 23 spores/ unit which highlights a level of infection of 5,750,000 spores per bee. Besides of the two experimental modules, in the trial was included also a control module, each module of two batches. The groups of bees were kept in wooden cages (with 190/ 150/ 50mm size) fitted with glass, ventilation mesh and feeding device, with at least 100 bees/cage (Figure 2). The tested bees had at their disposal food such as salubrious crystallized honey.

The trial was conducted in laboratory conditions, in a heated room at a temperature around 28 ºC and 28% humidity (Figure 3).

Figure 2. Bee cage used in the experiment

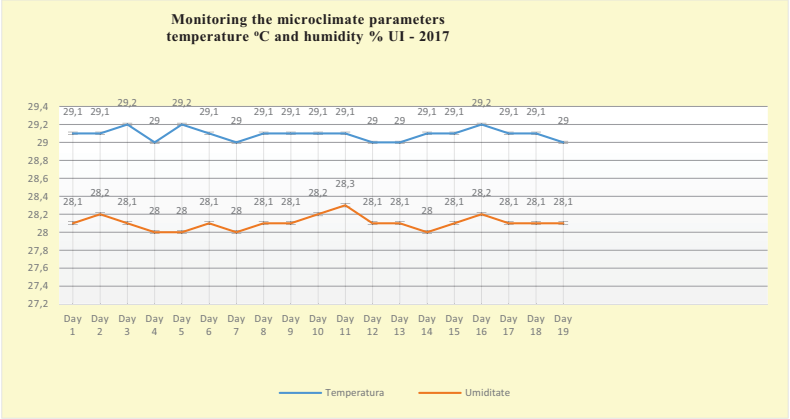

Figure 3. Monitoring the microclimate parameters (temperature and humidity) during the experiment

Amprolium hydrochloride 20% was administrated in 1/1 sugar syrup (60% SU), in an amount of 1g (5ml) per litre. The pH of the sugar syrup was adjusted with food vinegar to 3.5 - 4. The medicated syrup was administrated by spraying on bees, every day, such as: 0.2 ml (200ppm a.s.) (Module 1), respectively 1 ml (1 mg) (Module 2) (Table 2). For the control module, 1 ml of sugar syrup 1/1 (60% SU) was administrated.

During of the trial, daily, there were performed observations regarding the general condition of bees and data on clinical signs and mortality were registered; the dead bees were counted and eliminated from each batch. Samples of dead bees were collected for subsequently laboratory investigations in order to determine the level of infection with *Nosema* spp.

Table 2. Experimental design: the experimental modules and dose of medicated syrup

Module	**Batch**	**Syrup with Amprolium hydrochloride*** (dose)
1. Experimental	1A	0.2 ml (200ppm s.a.)
	1B	0.2 ml (200ppm s.a.)
2. Experimental	2A	1 ml (1 mgr s.a.)
	2B	1 ml (1 mgr s.a.)
3. Control	Control 1	1ml sugar syrup**
	Control 2	1 ml sugar syrup**

*administered only to the experimental modules
** without Amprolium

RESULTS AND DISCUSSIONS

The study regarding the potential of Amprolium hydrochloride product in controlling *Nosema* infection in bees, performed in laboratory conditions over a 19-days period, allowed us to highlight the suppressive effect of the product. Bee colonies from both experimental and control groups were examined daily, registering: the microclimate parameters, clinical status and mortality of bees. The results are presented in Table 3. In this respect, no spores of *Nosema* spp. were found at the end of the trial in the experimental modules (to which the product was administered in different doses), while for bees in the control batches *Nosema* infection was still present and with increased intensivity (from 23 spores / unit = 5,750,000 spores / bees to 31 spores / unit = 7,625,000 spores / bees).

The survival rate of bees in the three modules (the two experimental modules and the control one) was not influenced during of the procedures.

There were no significant differences between the two experimental modules, to which were given different doses; moreover, a 5 times increased dose did not show a negative influence on the analyzed bees. The survival rate of bees in the control batch, under laboratory conditions, during the test registered lower degrees than the survival period of the bees in the two experimental modules. Mortality of bee in the experimental module 1 occurred after 16-19 days, after 16-18 days for the experimental module 2, and after 15 days for the control module, respectively (Table 3).

Table 3. Level of infection with *Nosema* spp. and bee loss (number of dead bees) per day and per module

Day	Batches included into the present study					
	Control Module		Experimentale Module			
	Control 1	Control 2	Batch 1 A	Batch 1 B	Batch 2 A	Batch 2 B
Day 1						
Day 2						
Day 3						
Day 4	2					
Day 5	2	3				
Day 6	9	8		4		
Day 7	8	11	4	6	7	
Day 8	5	10	5	7	9	8
Day 9	8	10	12	13	11	7
Day 10	16	14	14	14	10	12
Day 11	13	19	15	12	14	14
Day 12	23	13	14	13	19	17
Day 13	21	18	15	12	19	19
Day 14	22	17	17	16	17	20
Day 15	13	16	16	17	20	23
Day 16			15	8	18	23
Day 17			18			22
Day 18			18			20
Day 19			22			
Total no of bees	142	139	185	122	144	185
Spores/bee	7625000	7625000	0	0	0	0

From the presented data, the Amprolium hydrochloride 20% has proven to be able to control the development of *Nosema* spp. parasite. In the experimental groups, at the

beginning of the trial, there was recorded an infection rate of 23 spores / unit, which means 5,750,000 spores / bee, while at the end of the experiment, for the experimental batches after administration of Amprolium hydrochloride, no spores of *Nosema* spp. were detected. There were no registered clinical signs or other adverse effects on bees. In the control group, the degree of infection increased from 23 to 31 spores / unit, which represents an infection rate of 7,750,000 spores / bees. In this respect, it is known that at an infection of 36 spores / unit, which highlights 9,000,000 spores/bee, the changes in the intestinal epithelial cells are of irreversible nature (Chioveanu et al., 2009).

Taking into account the manufacturer's recommendations for the use of the product in other species, the therapeutic dose for Amprolium hydrochloride was estimated at 10 mg/kg alive weight (equivalent to 10,000 bees), being administered daily for 7 days in 200 ml syrup, the dose of substance which was able of showing a therapeutic effect.

The therapeutic target for the use of amprolium hydrochloride as a drug is important because the host-parasite's metabolic pathways are common and the product blocks the thiamine absorption of protozoans, preventing the synthesis of carbohydrates. The product does not create resistance problems and no secondary phenomena have been observed. Long-term administration may result in thiamine deficiency (B1 vitamin). Thus, administration of the product should be performed only on a diagnostic basis. Also, due to the very rapid evolution of the disease, the duration of treatment should not last longer than 5-7 days in order to avoid the risk of drug interference with bee metabolism. In case of side effects, the treatment is discontinued and B1 vitamin should be given.

This experimental study was intended to be prospective, for testing and assessing a therapeutic dose. However, additional field studies, in the apiary are necessary. Analyzing the results, Amprolium hydrochloride 20% did not prove to have negative influence for the viability and vitality of bees even at a five times higher concentration than the maximum estimated therapeutic dose. Nonetheless, we admit that products used against nosemosis can stop the disease but without succeeding in the destruction of the spores in the hive. Disease in latent form may re-occur at any time following the emergence of favourable factors. Therefore, use of Amprolium hydrochloride together with effective prevention and control measures can solve this. For this reason, additional field and laboratory studies requires a development of an integrated pest control program on bees' Nosemosis.

CONCLUSIONS

Using the 20% Amprolium hydrochloride product to control nosemosis in bees under laboratory conditions, it was observed that it blocks the multiplication of *Nosema* parasites, preventing the clinical diseases. However, in order to develop an integrated control program for the prevention and control of nosemosis, additional field and laboratory studies are required.

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ERASMUS PROGRAM FOR VETERINARY FIELD AFTER ROMANIA'S JOINING THE EUROPEAN UNION: CHANGING LIVES, OPENING MINDS

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Abstract

After Romania has joined the European Union (EU) in 2007, the Erasmus program opened "new doors" for the academic community all around the county. Nowadays, the education and training are essential in achieving a better Romania, Europe and World. After 20 years of Erasmus program in Romania, celebrated last year, it can be seen the impact of Erasmus generation; former Erasmus students being now European Parliament members or governors in other countries. Morover, the longevity of this program (more than 30 years) makes the process of internationalization to be most commonly declared aspect of the Erasmus experience.

Incoming and outgoing persons data were collected for10 academic years, starting to 2006-2007, then it was used the interview technique for exponents from each group: outgoing undergraduates, outgoing PhD students, incoming PhD students and outgoing teachers.

The main purpose of this paper is to highlight the importance of the international cooperation in the mobility of persons generally, and of undergraduates particularly. This fact is revealed by the double acceptance Erasmus+ mobility: outgoing and incoming undergraduates.

Key words: *Erasmus program, Veterinary medicine, Romania's accession to EU.*

INTRODUCTION

The idea of student mobility as European integration model was initiated by Lijphart in 1964, but it started in June 1987 under the European Commission coordination as Erasmus program (Oborune, 2013). This program was named after the Dutch philosopher and catholic monk Desiderius Erasmus of Rotterdam (1466-1536), but, at the same time, Erasmus is an acronym meaning **Eu**ro**R**opean community **A**ction **S**cheme for the **M**obility of **U**niversity **S**tudents (Feyen and Krzaklewska, 2012).

At the beginning, the program involved 11 countries, with 3244 students travelling abroad for study stages. Since then, Erasmus program has undergone a series of major changes: increasing the number of involved program countries (more than 33), and including partener countriens (from other continents) (Krzaklewska and Krupnik, 2008; Vossensteyn et al. 2010; González et al., 2011; Feyen and Krzaklewska, 2012).

According to UNESCO statistics, about half a million students studied abroad in the early 1970s, about a million in the early 1980s, and about 1,5 million in the mid-1990s, as a result of Sorbonne Declaration (1998), Bologna Declaration (1999), and Lisbon Strategy (2000) (Otero, 2008).

Around 90% of European higher education institutions (more than 4,000) from 34 European countries are joining Erasmus. The proportion of students in the Erasmus program varies between 0.1% and 1.5% of all students enrolled, with the exception of Luxemburg where the participation rate exceeds 6% (Vossensteyn et al. 2010; Ballatore and Ferede, 2013).

Starting with 1995, Erasmus program became part of Socrates program, and the spectrum of its activities was gradually widened to include educators' mobility and international

cooperation among universities. After 11 years and, more than one million students that studied abroad, the Socrates-Erasmus program collaboration ended. EU replaced in 2007 the Socrates program with "Lifelong Learning Program" (LLP), that was designed to give the opportunity to people at any stage of their educational life (student placements in enterprises, university staff training, and teaching business staff) to develop their skills and training across Europe. Starting with 2014, the activities of LLP continue under the new Erasmus + program (Sigales, 2010; Jacobone and Moro, 2015).

Romania started the participation in the Erasmus program in 1998 (Stoika, 2013). Ten years later, 2.937 of outgoing Erasmus students were enrolled in this program, representing 14% from the total number of students abroad, but less than students enrolled in 2004 (2.960 students). In 2008, there were 863 incoming Erasmus students compared to 602 in 2005 that studied in Romania (Vossensteyn et al. 2010).

For this paper, our purpose was represented by the statistical analysis regarding Erasmus program in the Faculty of Veterinary Medicine of Bucharest, in correlation to Romania's joining the EU. Another aim was to look through our faculty students' and teachers' eyes in order to obtain an objective opinion regarding this program.

MATERIALS AND METHODS

The study was based on official data provided by International Relations Department of the University of Agronomical Sciences and Veterinary Medicine of Bucharest in 2017.

Official data of Erasmus program were collected in 2017 for a decade of academic years for the undergraduates, PhD students, and teachers of Faculty of Veterinary Medicine. Outgoing and incoming persons, framed as undergraduated students, PhD students and teachers were considered during the study period.

To find new results about the Erasmus program perception, a standard questionnaire was conceived for exponents from each group: outgoing undergraduateds, outgoing PhD students, incoming PhD students and outgoing teachers (table 1).

Table 1. The questionnaire for the Erasmus program exponents

Questions
1. Do you consider that the Erasmus program is sufficiently well promoted in our university/ faculty?
2. Do you consider easy to get an Erasmus internship?
3. Do you consider that the financial support is sufficient for this internship?
4. Would you like to have a double funding (Erasmus program and university)?
5. Do you think there are enough universities abroad to choose from for an Erasmus internship?
6. Do you consider the information gathered in this internship is useful?
7. Regarding the equivalence of ECTS after returning from the internship, do you think it is difficult?
8. You think you have been disadvantaged as a Romanian at the internship/mobility?
9. Do you think that Romania's entry into the EU has brought advantages to the Erasmus program?
10. Regarding the experience gained from this internship/mobility, would you recommend to other people to apply?
11. Would you repeat this experience? And if so, at the same center or another one?
12. Do you think you left a good impression after completing the internship?
13. Do you think that you had stimulated the curiosity of foreign students / teachers to follow a similar internship within our University?

There were 13 questions the subjects had to answer with "yes" or "no".

The number of people who coordinated this program in the faculty and the number of bilateral agreement were taken in consideration.

RESULTS AND DISCUSSIONS

After analysing the data of outgoing undergraduates during the last 10 academic years, it can be said that after 2007-2008 academic year till 2016-2017 academic year, the number increased from 1 to 4. During this 10 academic years, the total number of outgoing undergraduates was 42 (figure 1). The highest number of outgoing undergraduates was 6, between 2008-2009 and 2010-2011 academic years.

The incoming undergraduates started to come in 2010-2011 academic year, increasing progressively from 3 to 10 in 2015-2016 academic year. An essential mention is that all

the incoming undergraduates were from Italy. Regarding the incoming teachers, the number was more or less constant, starting with 4 in 2010-2011, to 5 in 2016-2017 academic year. A similar situation was observed for outgoing teachers, from 1 in 2006-2007 academic year to 2 in 2007-2008 academic year, but this number varied until 2016-2017 academic year.

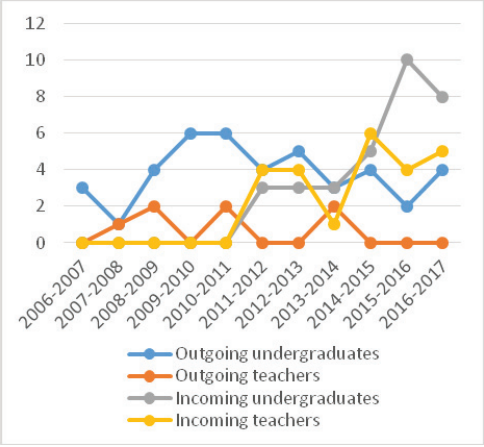

Figure 1. Graphical representation of outgoing and incoming students and teachers from 2006 to 2017

In 2016-2017 academic year it was a premiere for our university, when a Romanian undergraduate started as an outgoing Erasmus undergraduate, graduating in the outgoing country, then starting the PhD program in the same country and, finally coming in our faculty as incoming PhD student. Starting with 2003-2004 academic year up to present, just 3 persons were Erasmus program coordinators for our faculty.

At this moment, the Faculty of Veterinary Medicine of Bucharest has 8 bilateral agreements (Austria, Bulgaria, Italy, Poland, Portugal, Spain and Turkey).

Regarding the questionnaire's answers, probably the most interesting one is at the first question („Do you consider that the Erasmus program is sufficiently well promoted in our University/Faculty?"), where 75% of the exponents answered „no". At the question „Do you think that Romania's entry into the EU has brought advantages to the Erasmus stage?", half of them answered with „yes" (table 2).

For all exponents the financial support was not enough, a double founding being consedered a need. For all of them, the information gathered in this internship was useful, and for the future they would recommend to other people to apply for the Erasmus program.

Table 2. The Erasmus exponents' answers of the questionnaire

Question number	Undergra-duates outgoing	PhD students outgoing	PhD students incoming	Teachers outgoing
1	Yes	No	No	No
2	Yes	Yes	Yes	Yes
3	No	No	No	No
4	Yes	Yes	Yes	Yes
5	No	No	Yes	No
6	Yes	Yes	Yes	Yes
7	Yes	Yes	No	No
8	No	No	No	No
9	Yes	Yes	No	No
10	Yes	Yes	Yes	Yes
11	Yes/No	Yes/No	Yes/Yes	Yes/No
12	Yes	Yes	Yes	Yes
13	No	Yes	No	Yes

CONCLUSIONS

Following the above results, the first conclusion is that the number of incoming students is continuously increasing, while the amount of outgoing students decreased starting with 2009-2010 academic year. Another important conclusion is that the subjects didn't neglect the financial support. To correct these deficiencies, the University will double the places and the amount of money for outgoing students in 2017-2018 academic year. The highest number of incoming students was in 2015-2016 academic year, when 10 students came in our faculty to study. As a crowning achievement of this program, in the Faculty of Veterinary Medicine of Bucharest, it was implemented the English language program in 2013-2014 academic year.

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