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CONTENTS

THE ROLE OF PARASITIC INFECTIONS IN THE DEVELOPMENT OF RESPIRATORY DISEASES IN SWINE

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Abstract

Metastrongylidosis is a parasitosis caused by several species of nematodes of the genus Metastrongylus. The development of parasites goes through transitional hosts - earthworms. Infection occurs when pigs eat infected worms so infections are most commonly found in organic production and extensive breeding. The pathological effect of parasites begins with their larvae migratory movement from the lung capillaries to the lung tissue, during migration through the lung tissue, during the stay and activity of adults in the bronchi and aspiration of parasite eggs into bronchioles and alveoli. This is followed by the toxic effect of metabolic products of the parasite, which after resorption in the blood can lead to general intoxication. The predilection place of parasites is the posterior parts of the diaphragmatic lobe - margo acutus and margo obtusus. Affected animals show signs of dyspnoea and frequent vesicular respiration. In addition to the direct pathological action of metastrongylide, they transmit several diseases of pigs of bacterial and viral etiology. Two species of these parasites, *Metastrongylus elongatus* and *Metastrongylus pudendotectus*, have been identified in Serbia. The prevalence of both species varies from region to region. In the north of Serbia (Vojvodina), the presence of *M. pudendotectus* dominates, while in central and southern Serbia, *M. elongatus* is much more common. In Serbia, in individual (semi-extensive and extensive) housing, infections are found in 34-52% of animals and in 1-3% of swine in farms.

Key words: *Metastrongylus spp.,* swine, respiratory diseases

INTRODUCTION

With increasing demand for products resulting from organic farming and corresponding better animal welfare closer to natural behaviour, animals are increasingly kept outdoors grow posibility to have direct contact with numerous intermediate hosts of parasites and other diseases (Loskot V.I., *et al.* 1988; Pavlović I., *et al.* 2013,2017; Adedokun O.A., *et al.* 2001; Carstensen L.,*et al.*2002).

The dominant species of parasites in this breeding are biohelminths and lungworms are one of the most common. (Dunn D.R., *et al.*1955; Kruse G.O.W., Ferguson D.L., 1980; Pavlović I.,*et al.* 2012). *Metastrongylus* sp. infection is reported all around the world in wild boars and pigs (Barutzki D.,Richter R.,1980; Alcaide M.,*et al.*2005; García-González A.M.,*et al.*2013; Nagy *et al.*,2013; Spieler N.,Schnyde M.,2021).

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In Serbia, research on the Metastrongylus species in domestic and wild swine has been done periodically (Pavlović I.,*et al*. 1995, 1997, 2005b, 2011).

SYSTEMATICS AND DISTRIBUTION OF METASTRONGYLUS

All Metastrongylus are in the class Nematoda, subclass Myosyringata, order Strongylata, suborder Metastrongyloidea, family Metastrongyloidae, subfamily Metastrongylinae and genus Metastrongylus. A total of 6 species have been identified in this genus: *Metastrongylus apri* (syn. *M. elongatus*), *Metastrongylus asymmetricus, Metastrongylus confusus, Metastrongylus madagascariensis, Metastrongylus pudendotectus* and *Metastrongylus salmi* (Gasso, D., *et al.* 2014),

The geographical distribution of individual species varies so that *M.apri* and *M.pudentotectus* have the widest distribution. *M.confusus* is found in Europe including Poland and *M.salmi* has been found in South America, Europe and Africa (Khrustalev A.V., 1981; Drozdz J., Zalewska-Schonthaler N.,1987; Singh B.B., *et al.* 1989). The last two species, *M.tschiauricus* and *M.madagascariensis* have been described only in feral pigs, the first species in the Netherlands and Georgia and the second found only in Madagascar. Most of these species persist in feral pigs so that cross-infections occur in environments where extensive housing (packing) is present (Corwin R.M., Stewart T.B., 1992).

Two species of *M.elongatus and M.pudendotectus* have been established in Serbia. The prevalence of both species varies from region to region. The presence of *M.pudendotectus* dominates in Vojvodina, while *M.elongatus* is much more common in central and southern Serbia. (Pavlović I.,*et al.* 1997,2005b).

BIOLOGY AND MORPHOLOGY OF METASTRONGYLUS

The genus Metastrongylus is characterized by a thin long whitish body, the oral cavity is small and the oral opening is surrounded by two small three-lobed lips. The copolar bursa is relatively small with well-developed lateral lobes while the dorsal lobe is small (Soulsby E.J.L., 1977). At the time of laying, the eggs are embryonated and wrapped in a thin membrane that swells during their passage through the respiratory and digestive organs of pigs, so that we find eggs with a thick membrane in the feces.

EPIZOOTIOLOGY

Metastrongylides belong to biohelminths whose causative agents use transient hosts for their development and maintenance of the biological cycle, in this case numerous types of lumbricides (earthworms). Eggs are very resistant in the external environment and can remain vital in a humid environment for up to 2 years. Depending on the external conditions, larvae are released from the eggs, which can survive in the external environment for up to three months but are not infectious to the true host (Dun D.R., *et al.,* 1955; Kruse G.O.W., 1978; Vanparijs O., Thienpont D.,1982; Humbert J.F., Drouet J.,1990).

The larvae become infected only when they are eaten by earthworms - a transitional host. Depending on the geographical environment, numerous representatives of lumbricides persist as intermediate hosts. In environment condition in Serbia the dominant species of earthworms are *Eisenia foetida, E.rosea, E.veneta, Dandreobena rubida, D. octaedra, D.subrubicunda, Allopbophora caliginosa, A.jassyensis, A.longa,, Octolasium complanatuum, O.lacteum, O.rebeli, Lubricus terrestris* and *L.rubbelus* (Tričković D.,1978; Pavlović I.,*et al.*2005a).

In worms, the larvae are localized in the walls of the blood vessels of the esophagus and foregut. Here they hatch twice and develop into an infectious form in 10-25 days (Kruse G.O.W., 1978; Humbert J.F.,1992). After maturation, the larvae migrate into the blood vessels of the worms and remain infectious in them for up to 7 years. The larvae never leave the worms spontaneously (Ueno H., *et al.* 1960; Kruse G.O.W., 1978). Only in the case of earthworm damage (cutting during tillage, etc.) do they come out of the earthworm and from there reach the surface layers of the earth, where they can live up to 2 weeks, depending on the humidity.

ROUTES AND MODE OF INFECTION

Infection of pigs occurs orally. From the digestive tract, the larvae reach the mesenteric lymph nodes where they hatch. From there, they reach the bloodstream and lungs via the lymphatic system through the right heart. In the bronchi and bronchioles, the larvae grow and after 24 days reach the adult stage. The transparent period lasts 24-37 days (Dunn D.R., *et al.* 1955; Dunn D.R.,1957)

Young pigs aged 2-8 weeks are the most susceptible to infection. Maximum production of parasite eggs in the period of 5-9 weeks after infection. In the following period, the number of parasites decreases, but one number remains especially in the distal parts of the lungs (Kvachadze G.A.,1975).

PATHOGENESIS AND PATHOLOGICAL FINDING

The pathological effect of parasites begins with their larvae migratory movement from pulmonary capillaries to lung tissue, during migration through lung tissue, during stay and activity of adults in bronchi and aspiration of parasite eggs into bronchioles and alveoli (Dunn D.R., *et al.* 1955; Drozdz J., Zalewska- Schonthaler N.,1987). This is followed by the toxic effect of metabolic products of the parasite, which after resorption in the blood can lead to general intoxication.

The degree and intensity of pathological changes in the lungs directly depends on the intensity of the infection. In mild infections, the predilection site is the posterior parts of the diaphragmatic lobe - margo acutus and margo obtusus (Nakauchi K., *et al.* 1991, Ivetić V., *et al*.2000, Šabec D.,2002). Other parts of the lungs are also affected by severe infections (Marruchella G.,*et al*.2012). Bronchiolitis, bronchitis, diffuse pmeumonia, alveolar emphysema and connective tissue proliferation and cellular infiltration are observed (Ivetić V., *et al*.2000).

Some groups of lobules or groups of lobules are voluminous, grayish-white, and rustling (lobular emphysema) can be heard at the cross-section, and the changes are wedge-shaped, based on a bronchus

filled with parasites (Yoshihara S.,*et al.* 1990; Šabec D.,2002).

In bronchioles and bronchi, parasites are found in various developmental stages, either free in mucous exudate or surrounded by cellular infiltrate (Ivetić V.,*et al*.2003) (figure 1).

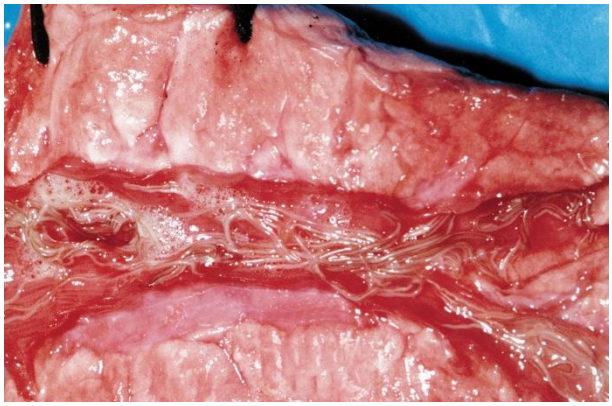

Figure 1. adult *Metastrongylus sp.* in bronci

In the pulmonary parenchyma, especially in the caudal part of the diaphragmatic lobe, gray nodules of 0.6-2 mm in size are found subpleurally, in which a central yellow or yellow-green field surrounded by a dark zone of connective tissue is observed (Nakauchi K.,*et al*.1991). This zone is composed of larvare of parasites, cellular infiltration of macrophages, eosinophils, lymphocytes and giant cells (Sasaki O.,Katsuno M.,1983;Ivetić V.,*et al*.2003)(figure 2).

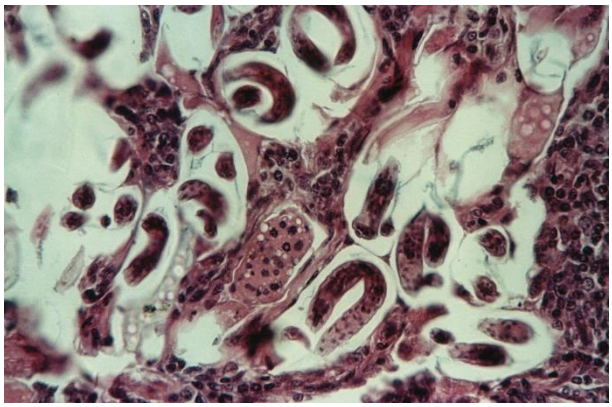

Figure 2. larvae of *Metastrongylus sp.* in the pulmonary parenchyma

CLINICAL SINGS

The clinical singns of disease depends on the degree of infection. In mild infections, they are weakly expressed - a weak cough is most often present. In severe infections, the symptoms appear as early as the second week after the infection, and are most pronounced 4-6 weeks when the disease progresses to a chronic stage. Diseased animals show signs of dyspnoea and frequent vesicular respiration (Pavlović I.,*et al.* 1997).

At the beginning, there is a weak and later a hoarse cough, which is in the form of an attack when the animals have stress (running, etc.). The mucous membranes are pale, appetite is reduced and eosinophilia is present in the blood. Body temperature is elevated only when secondary infections are present (Pavlović I., *et al.*1997). Metastrongylides are biological and mechanical vectors of many bacterial and viral infections in pigs. Influenza viruses and classical swine fever, which infiltrate the embryos of metastrongylide egg embryos and then persist in larvae in transient hosts and finally infect pigs that eat earthworms with larvae are certainly the most significant (Shope R.E., 1941, 1958, Pavlović I.,*et al*.2005b,2012). Pasteurellosis is also transmitted through metastrongylide and several other types of bacteria.

Thanks to that, the clinical picture of metstrongyld is additionally burdened with secondary infections that can be drastic and cause mass deaths of pigs (Corwin R.M., Stewart T.B.,1992; Pavlović I.,*et al.* 2005).

DIAGNOSTIC METHODS

Similar to other types of endoparasites, metastrongylides eliminate eggs in the environment through feces or sputum. In the feces of diseased animals, they are isolated by standard coprological methods (sedimentation, flotation), where the morphometric differences between individual species in this genus are very small, so it is most often mentioned in the finding as *Metastrongylus spp*. (similar to other strongylides) (Euzeby, 1981).

An autopsy report provides accurate information on the type of metastrongylide present. The morphometric differences are very clear so there are no problems related to the determination of the causative agent. Epizootiological data on the types of lumbricides and the finding of metastrongylide larvae in them is also an important diagnostic data that allows us to determine potential sites of infection.

THERAPY AND PROPHYLAXIS

In the control of metastrongylides, there is a large selection of preparations that are applied by food or injection. Neither has an effect on migratory larvae nor is it an ovocid. Metastrongylidosis is a

disease of pigs kept on pastures, in yards and in general on the outlets extensively or semi-extensively, although it will also occur in farm-kept animals in conditions when they are kept on the outlet. In the free keeping of pigs, the most important but also the least feasible preventive measure is the separate keeping of different age categories of animals. It is desirable to avoid contaminated pastures, and considering the length of life of earthworms (they live 2-7 years), it is also difficult to do, as well as avoiding mixing wild and domestic pigs, which is even more difficult when packing. Preventive deworming proved to be the most effective – autumn, which is done 3-4 weeks after withdrawal from pastures, and spring before expulsion to pasture. All animals must be treated (Pavlović I.,*et al.* 2005).

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THE RAT AND THE SHEEP, ANIMAL MODELS FOR THE STUDY OF PERIODONTITIS AND INDUCED PERIIMPLANTITIS OF BACTERIAL STRAINS SPECIFIC TO HUMAN ORAL MICROBIOTE

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Abstract

Periodontitis and periimplantitis are two diseases that have as a common element the progressive loss of alveolar bone, eventually leading to the loss of teeth and dental implants. The causes of the two diseases are multiple but the composition of the local bacterial biofilm is one of the important triggers. The aim of this review was to establish the main bacterial strains that can induce experimental periimplantitis and periodontitis as well as the techniques by which diseases can reproduce. The rat and the sheep are commonly used animal models in this branch of research because it reflects the main characteristics of human periodontitis or periimplantitis. The results obtained from the recent literature show that *Porfiromonas gingivalis, Aggregatibacter actinomicemcomitans, Streptococcus oralis* or *Fusobacterium nucleatum* (bacterial species commonly found in the human oral microbiota) are among the bacteria that can easily reproduce the two diseases of the oral cavity. Induction techniques include oral gavage, ligation technique, lipopolysaccharide injection, or the use of preinfected implant devices. The data accumulated in this review will be useful for research on the pathology of periodontal or periimplant diseases but also the approach of innovative therapies.

Key words: periodontitis, periimplantitis, rat, sheep, bacterial biofilm.

Periimplantitis is an osteolytic inflammatory disease (Koutouzis T. and colab., 2017) induced by a number of factors that result in orofacial implant failure (1-47%) (Sun J., 2014). This condition is a major topic of interest in the field of implantology because technical progress has been made, demand is growing, especially among the elderly population (Nickenig H.J, 2008, Passia N., 2017), and standardized therapeutic schemes for preventing and combating periimplant disease are still insufficient due to the uncertainty of the pathogenic mechanism involved. The pioneer of the dental implant is Per-Ingvar Branemark, who in 1978 presented the first dental devices in the form of titanium root (Brånemark P.I., 1986) thus demonstrating the possibility of osseointegration by bringing the implant into direct contact with the bone surface (Pătrașcu I., 2021). Over time, a wide variety of dental implants have been introduced to the market, each with the aim of better osseointegration and limiting rejection phenomena.

Periodontitis is a chronic immunoinflammatory disease of the periodontium that results in the progressive loss of gingival tissue, periodontal ligament and finally, alveolar bone (Pihlstrom B.L., 2005). This condition may be associated with host susceptibility (Schenkein H.A. 2006) but is primarily initiated by subgingival biofilms containing a gram-negative commensal microbiota and opportunistic pathogens, and the body responds by activating polymorphonuclear cells. They release destructive reactive oxygen (superoxide, proteinase) that destroys host tissue, eventually causing osteoclastic bone resorption (Chapple IL.C., 2002). In order to look for optimal treatment solutions, the implant must be differentiated from periodontitis, a condition with which it shares common characteristics (Mombelli A., 1995). Both describe an inflammation of the mucosa, increased depth of the gingival pocket, bone loss observable on radiographic examination and the presence of the bacterial biofilm (Lindhe J., 2008). As in periodontitis, the composition of the biofilm that develops in the pockets around the implant is dominated by gram-negative bacteria (Leonhardt A., 1999). Recent studies focus on the

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individualization of diseases and claim that there are significant differences in the composition of the biofilm (Dabdoub S.M., 2013) or on the body response, in the sense that inflammatory infiltrate of periimpantitis occurs around the implant, and periodontitis, plasma cells, macrophages and lymphocytes are found on the surface of periodontal teeth (Piattelli A., 1998, Lang N.P., 2011). Histopathological analysis of inflammatory infiltrate in periimplantitis shows that it crosses the bone barrier, migrating to the trabecular space (Lindhe J., 1992), as opposed to periodontitis, in which the inflammatory infiltrate is limited to soft tissues (Marinello, 1995, Ericsson, 1996, Persson, 1996, Gotfredsen, 2002).

Research on peri-implantitis has highlighted a number of factors involved including: shape, implant location, occlusal overload, time allotted to osseointegration, implant-abutment connection, release of metal particles (Zandim-Barcelos D.L., 2019) and last but not least pathogenic bacterial accumulation on implant surface. Since the early 1990s, experiments have sought to mimic periimplantitis based on the idea that bacteria are directly responsible for producing the phenomenon resulting in progressive bone loss (Lindhe J., 1992, Lindhe J., 2008), especially through the biofilms they develop on both the surface of teeth and implants. Biofilms can appear early, being dominated by species of streptococci and species of actinomyces (Kumar P.S., 2012, Quirynen M., 2002) which represent the substrate for additional bacterial attachment (Rosan B., 2000), and *Fusobacterium nucleatum*, the most common bacterium in dental plaque is the bridge with late biofilms characterized of bacteria such as *Treponema denticola or Porphyromonas gingivalis* (Kolenbrander P.E., 2010).

As the implantology industry is booming, it is necessary that the devices be evaluated preclinically both in vitro but especially in vivo. For this, it is crucial to find an animal model that helps to understand the triggering mechanisms of periimplantitis and that mimics the condition encountered among human patients. Various animal species such as rabbit, mouse, rat, guinea pig, dog, pig (Wancket L.M., 2014), sheep, goat or nonhuman primate have been included in biocompatibility studies or in experimental induction of periimplantitis. Ethical reasons, maneuverability, accommodation conditions, feeding conditions or clinical follow-up are essential elements in choosing the animal model, and today small animals such as mice or rats are preferred because they are genetically similar to humans in a 90%, and biologically and economically, is the best option. Of these two species, it seems that the rat is preferable because the mouse used in the research of dental implants did not provide sufficient clinical data to assess osseointegration or periimplantitis, in this animal model the oral cavity is poor in spongy bone (Yue G., 2020).

Sheep have many practical advantages over other animal models. Although sheep have become more widely used as experimental animals, there are not enough studies on their use for intraoral experiments. Instead, sheep are a popular animal model in bone research in recent years.

The aim of this review was to establish the main bacterial strains that can induce experimental periimplantitis and periodontitis in rats and sheep, but also the techniques by which the disease can reproduce.

MATERIAL AND METHOD

Search strategies

An electronic search for English language publications was conducted in June-July 2021, in PubMed / Medline, Web of Science, Google Scholar and Science Direct databases, in the search strategy using terms such as dental implant, periimplantitis, periodontitis, animal model, experimental periimplantitis, bacterial plaque, biofilm, sheep, rat. Inclusion criteria included experimentally induced periimplantitis, experimental periodontitis, rat / sheep as a model of periimplantitis or periodontitis, and all techniques and methods used to induce the two diseases. Studies aimed at inducing mucositis, animal models or induction sites other than the oral cavity were excluded from the search. For this review, 187 articles were analyzed, of which 42 (published after 2010 and until now) contributed to the collection of information of interest.

RESULTS AND DISCUSSIONS

The rat as a candidate model for periimplantitis

Rodents are the most commonly used animal models in biomedical research, and in the field of periodontology they have been widely used due to the many similarities with humans in terms of periodontal and histopathological anatomy (Sun J., 2020). Rats have the advantage of profitability, the ease with which they are manipulated and allow the standardization of experimental conditions in genetically similar individuals and human-like molar structure. They are suitable for the study of diseases related to the destruction and regeneration of tissues even if in terms of periimplantitis has the disadvantage of small animal size and continuous growth of dentition. Another disadvantage is that the microbiota of the rat is different from that of humans, their size is small and therefore the

amount of tissue analyzed is small, resulting in the need for a large number of animals (Helieh S., 2011).

The dental formula of the rat is I 1/1, C 0/0, Pm 0/0, M 3/3, and the incisor has no roots. This animal model is often used in experimental periodontitis, due to the periodontal anatomy of the molar region, which is very similar to humans (*Table 1*) (Yamasaki A., 1979). For example, the marsh rice rat (*Oryzomys palustris*) can develop periodontal disease from the age of 2 weeks (Helieh S., 2011), characterized by gingival inflammation, pocket formation, ulceration, alveolar bone resorption and tooth mobility, especially on the mandibular molars. Periodontal disease has been shown to be dependent on dietary factors, so soft, high-carbohydrate foods promote the disease among young animals, and a diet rich in protein and fat has reduced the severity of the disease (Helieh S., 2011, Shaw J.H., 1969).

Table 1

Similarities and histological differences of the oral cavity of the rat and human. (Listgarten M.A., 1975, Page R., 1982)

Similarities	Differences
superficial gingival bone and attachment of the junctional epithelium to the surface of the teeth	keratinization of the crevicular epithelium in rats
junctional epithelium appears to be a pathway for foreign substances, bacterial endotoxins and inflammatory cell exudates	the relationship between the gingival and junctional epithelium with desmosomal contact between the most superficial cells of the gingival epithelium and the non-keratinized cells of the junctional epithelium
	progressive change of the position of the molars in the three-dimensional space, resulting in the global movement in an occlusal-distal-buccal direction compared to the occlusal-mesial drift observed in humans
	the rat is resistant to periodontal disease
	weak inflammatory response in rats (neutrophils, few lymphocytes and an absence of plasma cells in the gingival tissues)

Sheep as a model of periodontitis and periimplantitis.

Their use has been reported in studies of critically sized bone defect models (Griffon, 2001), periodontal studies (Duncan, 2003), and techniques for augmenting facial bone / maxillary sinus (Haas, 2003). Their popularity is most likely related to their nature as higher-level vertebrates and their nonpet status. They are easily available, cheap to buy and maintain and respond well to surgical procedures (Salmon R, Duncan W, 1997). Disadvantages include difficulty in handling, requirement for large housing and lack of research information compared to other animal models. (An Y., Friedman R., 1999).

The dental anatomy of sheep differs significantly from that of humans. An edentulous area of 3 to 5 cm separates the mandibular incisors from the teeth of the cheek. The small and fragile mandibular premolars have a long and prominent hypsodont crown compared to the small mesial and distal root. Molar, premolar, periodontium and metabolic rate in sheep is similar to that in humans, as well as bone loss that occurs in sheep, on the age of aging (Vlaminck et al., 2008). The use of a sheep model in orthopedic infection studies was first reported in 1973, and since then they have been developed as models in chronic osteomyelitis studies. Orthopedic models using sheep and goats are well accepted, as their larger bone and spinal canal sizes allow the assessment of fasteners that would otherwise need to be modified for use in smaller animals, such as rabbits or rats (Stewart, 2012).

Sheep have a predisposition to periodontitis, a condition called “broken mouth“which according to microbiological research involves both local and disseminated bacteria in the rumen. The clinical manifestation is an acute one, most often associated with nutritional deficiencies. Diet has an important local effect on bacterial plaque formation and the development of periodontal inflammation. Vitamin deficiency, such as vitamin C, B12 and D, increases the prevalence and progression of periodontal disease, as well as reduced intake of magnesium, calcium, iron and zinc (Dommisch et al., 2018).

There is strong evidence to suggest that periodontitis is one of the factors leading to implant loss through the development of periimplantitis, and patients with periodontitis have a greater loss of dental implant and alveolar bone. The relationship between an oral biofilm-specific pathogen, the host response, and tissue destruction is a necessary first step toward the future goal of mechanistic studies under biofilm-mediated conditions, such as periodontitis or peri-implantitis (Lee D.W., 2014).

Numerous studies have attempted to identify pathogens associated with peri-implant infections. The methods were based on anaerobic cultures, microscopy, polymerase chain reaction, in situ hybridization of fluorescence or DNA-DNA hybridization, resulting in the detection of gram-negative, mobile cocci comprising *Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola* (red complex) also other species from *Treponema I to III* groups and *Synergistetes cluster A.* (Belibasakis G.N., 2016). In addition to these bacterial species, common in periimplantitis and periodontitis, *Peptostreptococcus spp.* or *Staphylococcus epidermidis* and *Staphylococcus aureus* were also identified, but only at the level of the implanted device. Comparative studies regarding the specific microbiota in periimplantitis have shown the presence of several genera such as: *Butyrivibrio, Campylobacter, Eubacterium, Prevotella (Prevotella nigrescens), Selenomonas, Streptococcus (Streptococcus nonmutan, Streptococcus mutans), Actinomyces, Leptotrichia, Propionibacterium, Peptococcus, Eubacterium spp., Lactococcus* and *Treponema*. Periimplant crevicular fluid analysis detected species of *Acinetobacter, Micrococcus and Moraxella* (Freire M.O, 2011, Belibasakis G.N, 2021).

Models of induction of periodontitis and periimplantitis

Techniques for inducing periodontitis or periimplantitis in rats involve inoculating specific pathogenic bacteria by oral gavage, intraoral injection or by placing ligatures around teeth or implants. The difficulty of reproducing the diseases occurs when the bacteria are applied in a growth phase incompatible with the formation of biofilm, especially when the indigenous flora also intervenes.

The "ligature-induced" defective pattern is commonly used to initiate periodontitis and periimplantitis in rats. Thus, a silk thread impregnated or not with pathogens is placed around the implant or tooth. Placement of a ligature leads to the accumulation of dental plaque and microulceration of the sulcular epithelium which, in turn, facilitates the invasion of periodontal pathogens into the connective tissue. Loss of periodontal attachment and resorption of alveolar bone occurs predictably over a 7-day period in rats (Nowotny A., 1983, Bezerra M.M., 2000, Bezerra M.M, 2002, Lohinai Z., 1998, Xie R., 2011). The role of bacteria in this model is supported by the findings that osteoclastogenesis and alveolar bone resorption are improved by the application of gram-negative bacteria (Lohinai Z., 1998). However, ligament-induced traumatic injury is limited only to study the pathological mechanisms of human peri-implantitis (Klausen B., 1991).

The lipopolysaccharide (LPS) application model was used to examine innate immune hosts using either LPS injection into the gingival tissue or LPS into the gingival scroll. The lipopolysaccharide component (LPS) of the cell wall of gram-negative bacteria is a significant inflammatory stimulus that triggers an innate immune response. The commonly used injection site is the palatal appearance of the first upper molars, but some studies have also performed injections on the interdental papilla between the first and second lower molars (Dumitrescu A.L., 2004, Nishida E., 2001). In rodents this pattern causes severe inflammatory responses of the peri-implant tissue and significant bone loss. Despite the sensitivity and accuracy in inflammatory induction, the use of LPS in the induction of periodontitis or periimplantitis is not similar to human disease due to the lack of bacterial colonization.

The rodent model that uses pre-infected implants, which investigates the host's responses against titanium implants on the surface of which bacteria form biofilm (Freire et al., 2011).

Infection model with *Aggregatibacter actinomycetmcomitans* (*AA*)

Rodents, although preferred as a model for inducing periodontal disease or periimplantitis, have the disadvantage that bacteria used to induce the disease process only temporarily infect the oral cavity, as rodents are not natural hosts for many human bacteria. A well-documented exception to this general principle is infection of the rat with *AA* which naturally colonizes the oral cavity. It has been hypothesized that *AA* may form a biofilm on titanium implants, which in turn can be used as a colonizing substrate for other bacterial species. Aa is common to periodontitis and periimplantitis, easily forms a biofilm on implants, and in rats, they lead to clinical reproduction of the disease, from tissue destruction to osteolysis.

The wid type of *AA* adheres to rat mouth epithelial cells, is frequently found in rice rats, while Sprague Dawley rat is difficult to detect, although it can also colonize it (Fine D.H., 2005).

The *AA* model was also used to examine periodontal bone resorption and the host's systemic response to infection. Li et al (Li Y., 2010) examined the role of T, B and CD4 + cells in adaptive immunity resulting in increased lymphocyte counts in regional lymph nodes as well as high levels of IL-2, IL-1, TNF, CD40 ligand, FasL, RANKL and osteoprotegerin.

Oral gavage model

The introduction of human bacterial strains by oral gavage and the subsequent impact on the periodontium has been studied in different rodent models (48). Various bacterial strains associated with periodontitis in humans have been used in this model, including *Porphyromonas gingivalis*, AA, *Tannerella forsythia*, and *Treponema denticola* (Garlet G.P., 2006, Sharma A., 2005, Lee S.F., 2009, Kesavalu L., 2007, Okada Y., 2010). Rats are usually given a known number of bacteria in a viscous suspension (2% carboxymethylcellulose) administered orally. Although the infection is transient, 45% of rats exposed to *Porphyromonas gingivalis* and 80% exposed to *Treponema denticola* or *Treponema forsythia* were found to harbor these bacteria after 4-6 weeks. Significant bone loss can be measured histologically, by macroscopic analysis or by computed tomography. Alveolar bone resorption is usually assessed around the maxillary molars, because the induction of bone loss in the lower molars is slower due to the thicker cortical alveolar bone and wider bucolic dimensions (Polak J., 2005).

Oral infection by topical administration of bacteria was also performed in rats. Many of these studies examined the Sprague Dawley strain (Lazar V., 2017).

One aspect that has been discussed in the oral infection model is the use of a single bacterial species versus the use of two or more microorganisms associated with periodontal disease. The complexity of bacterial stimulation is supported by the findings that the persistence of *Porphyromonas gingivalis* in the oral cavity of rats at 4 weeks after the initial challenge is significantly increased from 45 to 80-100% when this bacterium is co-infected with *Treponema forsythia* and *Treponema denticola*. Alveolar bone loss is significantly higher in animals caused by a polymicrobial oral infection than by monoinfection (Lazar V., 2017) but careful analysis of recent studies shows that periodontitis is induced by *Porphyromonas gingivalis*, compared to periimplantitis in which the polymicrobial biofilm is preferred.

If in terms of rat as a model of periimplantitis and periodontitis there are numerous studies, in the literature for sheep, the results are very poor, despite their similarity to humans, in terms of bone. Very few studies have used sheep as a model of periodontitis using the ligation technique mainly (Alexandru el al., 2019). Bacteria with implication in the study of periodontitis experimental sheep are represented by species of *Prevotela* (*Prevotella buccae, Prevotella intermedia, Prevotella loescheii, Prevotella melaninogenica*) and *Porphyromonas* (*Porphyromonas asaccharolytica, Porphyromonas endodontalis, Porphyromonas gingivalis, Porphyromonas gula*) (Borsanelli et al, 2017). The oral microbiota of ovine periodontitis is compatible with that found in human periodontitis. A study conducted by Silva et al., 2019, showed that the most common microorganisms in sheep with severe periodontitis were *Tannerella forsythia, Treponema denticola , Fusobacterium nucleatum and Porphyromonasgingivalis* while *AA , Enterococcus* gum were detected in none of the samples analyzed.

As in the case of the rat, the induction of periodontal disease is most easily achieved with the help of the *Porphyromonas gingivalis* strain that caused epithelial infiltration, collagen decomposition and bone resorption similar to the degenerative processes specific to human periodontitis. In addition, significantly higher levels of IgG antibodies against *Porphyromonas gingivalis* antigens have been observed in sheep with periodontitis, levels similar to those in humans (Genco, 1998).

In current research, based on keywords, no studies of experimental periimplantitis in sheep have been found, with bacterial implication which means that this animal model is still unexplored in this field.

CONCLUSIONS

Porphyromonas gingivalis is one of the most important periodontal pathogens, which has the ability to adhere to and invade the epithelial tissue of the oral cavity in both rats and sheep. *Aggregatibacter actinomycetemcomitans* can easily colonize the rat's oral cavity and titanium implants. *Fusobacterium nucleatum* is an important periodontal agent, especially in forms of rapid and progressive periodontal disease. *Prevotella intermedia* is pigmented in black, while *Bacteroides forsythus* is a non-pigmented gram-negative bacterium. These bacteria produce pro-inflammatory lipopolysaccharides and extracellular proteases that could destroy IgA immunoglobulins. *Microseptopreptococcus spp* has been positively associated with dental implant failure. Spirochetes (*Treponema vincentii* and *Treponema denticola*) have been observed to a greater extent in patients with periodontal disease than in healthy individuals and are capable of producing pro-inflammatory lipopolysaccharides and unusual metabolic products such as indole, hydrogen sulphide and ammonia, which are potentially toxic to host cells.

Therefore, used alone or in combination these bacteria can reproduce both periodontitis and periimplantitis in rats. This model reflected the

main characteristics of the two human diseases and may be a useful tool for future research into the relevant pathological pathways of peri-implant diseases, as well as for new therapeutic approaches.

Due to the similarity with humans in terms of bone structure, size, common oral microbiota and susceptibility to periodontitis, sheep can be an animal model for the study of periimplantation.

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CONTRIBUTIONS TO THE INTERPRETATION OF MICROBIOLOGICAL RESEARCH ON THE MICROFLORA OF DIFFERENT FISH VARIETIES

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Abstract

The scientific research reflected in this study aimed to identify the bacterial microflora in different varieties of fish of different marketing categories through microbiological investigation. The microbiological conditions for assessing the examined fish assortments determined the presence of saprophytic germs, asserting a normal microflora according to the requirements of the microbiological investigation standards and the identification of existing microbial species.

Key words: Fish, Bacteria, Chickens, Salmonella, Food Toxin Infections, Food Safety.

The animal-food-human relationship has become of unprecedented importance and impact, given that the 21st century has brought globalization and agro-industrialization to the world and, consequently, new challenges in developing an optimal model for a functional and balanced diet.

Primary production, processing and trading of food in the face of declining risks are a major priority, due to the profound implications that food and nutrition have on the lives and health of consumers. Food is the most conducive vector of multiple biological, chemical or physical risks, as well as important nutritional problems, so the consumer is more concerned about how he eats and has the desire to eat as healthy as possible to prolong the life [2,7] .

In this context, a special food due to its nutritional content and special taste qualities is fish, considered one of the most valuable food products due to easily assimilable nutrients necessary for human life, which contains: proteins, vitamins, minerals, enzymes, etc. [1,4] .

The action of microorganisms on fish as food can be variable and can influence the physico-chemical, nutritional and organoleptic characteristics. Microbial activity is most often manifested in connection with enzymatic reactions. It must also taken into consideration that micro-organisms may also intervene during the formation of the raw material. Microorganisms in the fish industry have a special role by modifying the organoleptic and nutritional properties of fish, which due to its structure is a beneficial environment in the development of microorganisms[3,6].

For these reasons it is important to know the microbial pathogens, which pollute the fish and contribute to its degradation [5,8,10].

At the same time, it is known that microorganisms that act harmful on food, generally making them unfit for human consumption are: bacteria, molds, yeasts that produce various processes of fermentation, mold and rot [9].

Studying various bibliographic accounts of different authors, I found it appropriate to conduct some scientific researches in this field and for this reason I aimed to identify the bacterial microflora in different varieties of fish of different marketing categories by investigating and identifying the existing microbial species.

MATERIAL AND METHOD

In order to perform out the study, investigations were performed according to the classical bacterioscopic and bacteriological laboratory methods of some varieties of Crap, Mintai and Hec fish purchased from the central square and the market from Chisinau municipality.

RESULTS AND DISCUSSIONS

The detailed analysis of the research allowed us to find and analyze the microbiological aspects based on the detection of the number of pollution microorganisms studied by their morphological, cultural activity, mode of action and other properties that are particularly complex and important. Organoleptic examination of fresh and frozen fish assortments was performed according to the following organoleptic indicators: muscle stiffness, appearance of the mouth, eyes, gills, skin and scales, nose, muscles (on the fish as such and on the surface of section) and assessment of the appearance of the viscera. In

the case of frozen fish, the examination was performed after defrost.

Organoleptic research of the fresh fish from the Crap assortment confirmed the presence of the muscular rigidity, the mouth closed, the appearance of the eyes was at the level of the orbits, the gills were reddish, without a characteristic smell, no characteristic mucus was observed. The appearance of the skin and scales showed a shiny natural color, the scales were a little shiny, they were well attached to the skin, and there was a small amount of mucus on the surface. As for the muscles, elasticity was observed, it was well attached to the bones, gray in color, white in pink. The viscera were well examined and individualized, with a specific smell. However, these researches lead to the conclusion that the fish investigated from the Crap assortment was in a state of first freshness according to the results of organoleptic investigation.

Organoleptic research of the Mintai and Hec frozen fish assortments presented characteristic organoleptic aspects through the following organoleptic indicators: slightly ajar mouth; exophthalmic eyes; slightly shiny scales and slightly shiny skin. These aspects allowed us to deduce from the fact that the assortments of frozen fish that were purchased from the market are of relatively fresh origin.

The microbiological researches regarding the qualitative microbiology of freshness of fish assortments of different categories reported indices differentiated according to several aspects of fish investigation. Thus, according to the specialized bibliographic information of food microbiology, it is considered that the microbiological analysis of the investigation of the freshness of the fish food product, appreciates this food product according to the number of microorganisms that pollute it. Therefore, it is considered that if microscopic field smears of fish fingerprints harvested from the surface layer are observed single saprophytic microorganisms cells (shells, rods), then this assortment of fish is considered the category of product of the degree - first freshness.

In the same time, if on the smear fingerprints under the microscopy of the fish samples are listed from 10 to 30 saprophytic cocci on the surface layer, then the fish is considered fit, fresh and allowed to be used in food. In the deep layer of fresh fish must contain unique insignificant microbial cells 1-2 saprophytic cells in the immersion field. Also, the specialized bibliographic sources of fish microbiology inform us that it is forbidden to use fish in food in order to prevent food poisoning, if as a result of bacterioscopic and bacteriological investigations of the surface layer of the fish to be examined was detected under microscopy from 40 and more microbial cells, and in the deep layer of the fish to be researched, more than 10 microbial cells were listed in the microscopy field.

Microbiological investigations of fish of different varieties Crap, Mintai and Hec aimed to evaluate the bacterial microflora in this food product through microscopic investigations on microbial preparations on the enumeration of the total number of germs in the superficial and deep layers of this food and assessing the quality of its freshness.

Following the data of the number of germs on the microscopic fingerprints of the Fish Carp assortment (Table 1) we deduce that the degree of pollution of the surface layer microflora constitutes 8 bacterial cells in the form of unique cocci, chaotically isolated, Gram positive. The deep layer of the Crap fish assortment shows us that the bacterial microflora as a result of visualizing the microbial preparations is smaller, constituting 3 unique cocci cells isolated by bacteria.

Therefore, according to our research study on this assortment of fish that we investigated after laboratory microbiological conduct, the following assessments follow that show that both the microflora of the surface layer and the deep layer of Crap fish meet the requirements of microbiological analysis and standards, and this assortment of fish is a food product in the category of fresh state according to the quality of the fish.

Table 1

The quality of the freshness of the fish assortments regarding the quantity of the bacterial microflora

Assortment	Bacterioscopy/surface layer	Bacterioscopy/deep layer	Microscopy
Crap	8	3	Isolated Cocci, Gram+
Mintai	14	5	Isolated Cocci, Gram+
Hec	21	7	Isolated Cocci, Gram+

Table 2

Quantitative aspects of microbial colonies regarding the quality of freshness of fish assortments

Assortment	Bacterioscopy/surface Agar/Endo	Bacterioscopy/deep Agar/Endo	Bacterioscopy/surface/ deep tubes	Cultural characters
Crap	18/0	6/0	6/2	Grey/white colonies
Mintai	10/0	9/0	7/4	Grey/white colonies
Hec	18/0	15/0	12/9	Grey/white colonies

The fish of the Mintai assortment, according to the studies of the microscopic visualization of the total number of germs, confirms a higher number of microscopic bacterial cells, characteristic of the surface and depth layers, which constituted 14 and 5 bacterial cells from the cocci category. Microorganism sticks were not viewed. And yet, I want to emphasize that the surface microflora of Mintai is more enlarged due to some aspects related to the ways of keeping the fish in the store where it was purchased. Regardless of these storage aspects, however, this Mintai fish assortment meets the marketing requirements, because the allowable norm of microbial cells in the microscopy field is 10-30 cells in the microscopic field. Therefore, the Mintai fish assortment used in the diet does not present a danger of food poisoning and we can classify it as a relatively fresh food product.

The data in Table 1 confirm the bacterioscopy investigation of germs from the fish food mark of the Hec assortment, reporting a higher number of microorganisms -21 chaotically isolated cocci microbial cells isolated in the surface layer and 7 cocci cells in the deep layer of this foodstuff. The reports demonstrated above confirm us after differentiating from other fish species examined Carp and Mintai, that however the bacterial microflora visualized under microscopy according to the total number of germs in the Hec assortment is higher in terms of surface layer and deep examination layer. However, these issues are considered normal, given the requirements for the marketing of
fish food. Indices 21 and 7, which correspond to the microscopic aspects of the Hec fish assortment, correspond to the microbiological standards. The fish from the Hec assortment is considered less fresh, but does not present a danger to the health of consumers, because its rainfall is determined by saprophytic coccic microorganisms.

Microbiological aspects through the passages of the three varieties of fish Crap, Mintai and Hec reflect different aspects in Table 2, which confirms the bacterial microbiological data of the number of bacterial colonies listed on Petri dishes with simple and special culture media, their characteristic visual and cultural interpretation specific to aspects of specialized bibliographic conduct.

The interpretation of the results, regarding the quantitative aspects of the microbial colonies highlighted after the passages from the Crap fish assortment on the simple and special culture media on plates showed, that the quantitative number of the developed colonies is visually differentiated.

The data obtained allow us to deduce that the surface layer of the Crap assortment is contaminated with a number of 18 microbial colonies that developed on the agar / plate medium and 6 colonies that developed on the agar / tube medium, compared to the layer deep bacteriological investigation, which noted 6 colonies on agar / plates and 2 colonies on agar / tubes.

On the special Endo medium, the development of colonies specific to the development on this culture medium was not highlighted.

However, these aspects of the investigation show that the number 18 microbial colonies is not alarming, because it meets the requirements of microbiological behavior, especially as mentioned earlier in the subject of microscopic research, that no pathogenic bacterial cells were detected and in in this case we did not notice and no development was confirmed on the medium.

The Mintai fish assortment regarding the number of microbial colonies shown in the table allow us to confirm as a result of the enumeration of cultural aspects a number of 10 colonies and 9 colonies regarding the microflora on the agar medium on plates regarding the surface and deep layers of this fish assortment with an aspect of development of the cultural characters of light / white colonies and absence of development on the Endo environment of microorganisms specific to the pathogenicity of some characteristic microbial species. The results of the bacteriology of the tube passages imprinted 7 and 4 characteristic colonies on the agar / tube medium at the corresponding surface and deep layers, which indicates that in comparison with the number of colonies of this fish assortment is increased compared to the Crap fish assortment and confirms that the freshness is relative, but still corresponds to the aspects of microbiological requirements.

In this context, however, it must be taken in consideration that this assortment was procured in the frozen state and in order to be microbiologically researched as required, it was defrosted. Possibly the freezing process was long and in this way the physiological processes of the fish meat were slightly degraded, giving it an uncharacteristic pollution because according to the microbiological requirements the fish of the Mintai assortment corresponds to be used for the consumer.

The information regarding the bacteriological conduct of microbiological investigation of the Hec assortment according to the microbiological conditions shows us that this assortment of Hec fish compared to the Crap and Mintai fish assortments is more polluted with the microorganisms of the microbial colonies. Therefore, analyzing the number of colonies listed on plates and tubes with the appropriate culture media where the cocci species are entrained, as we saw under microscopy, the highest number of colonies is observed: 18 and 15 on the surface and deept layer on the plates Petri of the fish researched from the Hec assortment.

The bacteriology of this assortment of fish, regarding the passages in tubes determined in the examined layers 12 and 9 colonies. Therefore, these results confirm that this category of fish is older and not fresh.

Previous reports conclude that the Crap fish assortment is of the first freshness according to the number of colonies developed in the examined layers characteristic of this food product, followed by the Mintai fish assortment with a relative freshness and finally the Hec fish assortment with a dubious freshness, due the higher number of microbial cells visualized on the microscopic fields and the higher number of microbial colonies developed on the usual and special culture media. Possibly the larger number of colonies corresponds to unhygienic conditions for keeping the Crap assorted fish until it is sold in market conditions.

The cultural characteristics of the cultures developed after the passages performed correspond to the respective characteristics of gray / white colonies on the agar medium both on plates and in tubes and aspects characteristic of the development in the broth medium in the form of sediment and turbidity.

The laboratory conduct on the microbiology of different types of fish meat also aimed to identify coliform germs, salmonella and staphylococci in fish, which frequently cause food poisoning.

Microbiological laboratory determinations were performed to investigate Salmonella microbial agents. The germs of the suspicious salmonella colonies were investigated from the fish samples to be investigated, subsequently there were passages on the special culture medium Endo and simple media agar and broth. Bacteriological preparations were stained according to Gram according to the classical staining method. Salmonella bacteria were not confirmed on microscopic visualization and also no salmonella cultures were determined on the culture media when performing the passages. Therefore, all categories of fish to be investigated did not confirm the presence of Salmonella and according to the microbiological conditions these fish varieties meet the requirements. Samples of fish also did not determine Escherichia microorganisms on common and differential culture media. The colonies characteristic of this species, which show the presence of E.coli, were not formed on the Endo culture medium. That is why knowing the microorganisms in the fish industry is important to know the changes in the organoleptic and nutritional properties of the fish, which due to its structure is a beneficial environment in the development of microorganisms. For these reasons, it is important to know the microbial pathogens, which pollute the fish and contribute to its degradation.

These scientific interpretations deduce and ensure that food safety is presented by not affecting the health of the consumer, and fish meat due to its varied chemical composition and rich in the main groups of nutrients needed by the body: proteins, fats, carbohydrates, minerals and vitamins. etc., favors the normal functioning of the human body.

CONCLUSIONS

1. The microbiological conditions for assessing the examined fish varieties determined the presence of

saprophytic germs, asserting a normal microflora according to the requirements of the microbiological investigation standards.

2. The Crap fish assortment confirmed the smallest number of saprophytic cocci microorganisms both in the surface layers and in depth, ranking the freshest fish meat.

3. The Mintai and Hec fish assortments revealed a variable bacterioscopic and bacteriological number of saprophytic microorganisms, classifying the fish meat of these assortments of relative freshness.

4. The approach of the given topic regarding the assortments of fish sold in squares and markets according to the microbiological conduct reveals us that all categories of fish due to their dietary and nutritional values are edible, useful and can be used in food.

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MICROBIOLOGICAL RESEARCH ON THE DETERMINATION OF ANTIBIOTICOSENSITIVITY OF PATHOGENS IN ABCESSES OF SLAUGHTERED PIGS

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Abstract

The scientific research reflected in this study aimed to study the behaviors of the sensitivity of some bacterial species highlighted by the abscesses of pigs slaughtered against antibiotics and the interpretation of the microbiological aspects that define them. The antibiogram results of microbial strains isolated from samples of Escherichia coli, Staphylococcus aureus and Streptococcus pyogenes abscesses were evaluated in antibiotic substances, determining various sizes of susceptibility areas.

Key words: Abscesses, Microbial species, Antibiotic sensitivity, Antibiotic substances.

It is scientifically known that microorganisms tend to resist any antibiotic and this vision is a global phenomenon that affects huge territories. For these reasons, the characteristics of the resistance phenomenon vary depending on the relationship with the affected bacterial species, the range of antibiotics used, the distribution of resistant strains in certain places where antibiotics were used and the antibiotic resistance phenotype established by comparing active antibiotics to strains. reference, belonging to bacterial species (which may have natural resistance to certain antibiotics), with antibiotic substances to which the tested strain is resistant [3,10].

Antimicrobial resistance is considered to be the ability of microorganisms of certain species to survive or increase in the presence of a given concentration of an antimicrobial agent that is usually sufficient to inhibit or kill microorganisms of the same species[1].

The Study of Monitoring Antimicrobial Resistance Trend (SMART) is a global antibiotic resistance surveillance program that is ongoing and monitoring the susceptibility of Gram-negative and Gram-positive bacteria from intra-abdominal infections since 2002 [2,4].

That is why epidemiologically the resistance of bacteria to antibiotics varies from one geographical region to another, from one specialty to another specialty, from one type of infection to another, from one year to another and therefore we considered that the study of the resistance of some bacteria, which are involved in infections of different origins is an important and actual issue [6,7,8].

Microbial abscesses are common in medical and veterinary clinical medicine. The microorganisms Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes etc. are the most commonly isolated pathogenic microbial agents in these infections.

These aspects often have a cause or a factor that favors many mechanisms in the disorder of known physiological processes. When these mechanisms are affected by a condition, abnormality or trauma, they can no longer play this role of defense and bacterial infections can develop [5,9]

Therefore, the study of microbiological aspects in the case of these diseases is primarily due to research on antibiotic resistance in these bacterial infections and is motivated by the fact that such research is necessary to determine the level of resistance and the trend of this resistance.

MATERIAL AND METHOD

The scientific researches were performed in the Microbiology Laboratory of the Department of Food Safety and Public Health of the Faculty of Veterinary Medicine of the State Agrarian University of Moldova.

RESULTS AND DISCUSSIONS

The behaviors of 3 microbial strains identified from abscess samples were tested from

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pigs slaughtered against various antibiotics. To study the resistance to antibiotics, investigations were performed to determine the sensitivity of microorganism strains using the diffusimetric method.

Scientific research has determined the resistance of the following microbial strains identified from abscesses of slaughtered pigs: Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes. The assessment of the resistance of microbial strains to antibiotics by the method of washers aimed at making passages on agar plates of microbial cultures from liquid media.

For this purpose, 6 washers soaked with various antibiotic substances were applied on the surface of the media. The plates were incubated in a thermostat for 16-17 hours at t -37 ^{o}C. After the expiration of the time on the examined plates, the zones of inhibition of the microorganisms of the bacterial strains around the washers were measured. The dimensions of the zones depended on the degree of sensitivity of the strains to the corresponding antibiotic: Intermediate (I); Resistant (R); Sensitive (S). The aspects of determining the resistance of microbial strains denote practical importance, noted by topicality and new pathogenicity criteria. The determination of the chemoresistance of germs thus acquires a wider significance than a simple analysis for the choice of a treatment and is therefore debatable, regarding the technique of execution and interpretation of the results from several points of view.

The results of the antibiogram of the microbial strain Escherichia coli shown in Table 1 show important results of the sensitivity of antibiotic substances demonstrating various areas of sensitivity. The most effective antibiotic substance turned out to be ceftazidime - 277mm, followed by amoxicillin - 26mm, cefaclor - 25mm, etc.

Some specialized sources confirm that E. coli is responsible for purulent infections manifested by abscess. Among E.coli strains there are considerable differences in the repertoire and expression levels of virulence factors that may affect bacterial growth and persistence in abscess pathology. Therefore, these interpretations confirm the mechanisms of Escherichia coli resistance and sensitivity to antibiotics, which translates into the ability of a microorganism to survive in the presence of antibiotics or antibacterial chemotherapeutics.

According to this study, bacterial resistance can be genetically genotypic or phenotypic and therefore chromosomal genetic resistance occurs as a result of mutations in the nucleotide sequence of the bacterial chromosome, which causes the synthesis of proteins or other macromolecules, different from the initial chemical structures, so the action antibiotic can no longer be achieved, representing 10% of the acquired bacterial resistance.

The most important transporters for the transfer of resistance genes from one bacterium to another are plasmids, transposons and integrators. Genes that confer resistance are transferred from one bacterium to another in a horizontal manner by conjugation, transduction, or transformation.

Important scientific evidence confirms the results of the antibiogram of the uropathogenic microbial strain Staphylococcus aureus to the antibiotic substances listed in Table 2, which determines aspects of the areas of sensitivity of the tested antibiotic substances.

Important data revealed the highest degrees of sensitivity to the following antibiotics: amoxicillin-28mm, followed by cefazolin-26mm, ampicillin-24mm.

Resistance to some antibiotics, however, is associated with changes in the enzymes that neutralize the antibiotic. It is the result of the expression of enzymes that covalently modify these antibiotics by acetylation using aminoglycoside-acetyltransferases, phosphorylation by aminoglycoside-phosphotransferases or adenylation by aminoglycoside-adenylyltransferases.

Table 1

The sensitivity of Escherichia coli to antibiotics

Antibiotic substances	Degree of sensitivity		
	Resistant (R)	Sensitive (S)	Intermediate (I)
Doxacycline	0	22	0
Cefoperazone	0	20	0
Ampicillin	0	21	0
Ceftazidime	0	27	0
Amoxicillin	0	26	0
Cefaclor	0	25	0

Table 2

Sensitivity of Staphylococcus aureus to antibiotics

Antibiotic substances	Degree of sensitivity		
	Resistant (R)	Sensitive (S)	Intermediate (I)
Ampicillin	0	24	0
Cefazolin	0	26	0
Amoxicillin	0	28	0
Doxacycline	0	22	0
Celosporine	0	20	0
Oxacillin	0	21	0

Table 3

Sensitivity of Streptococcus pyogenes to antibiotics

Antibiotic substances	Degree of sensitivity		
	Resistant (R)	Sensitive (S)	Intermediate (I)
Ampicillin	0	24	0
Cephalosporin	0	22	0
Ceftazidime	0	20	0
Ofloxacin	0	26	0
Cefeprim	0	28	0
Amikacin	0	21	0

These enzymes are usually plasmidically encoded, but transposable elements may also be involved. Different bacterial phenotypes from various species can also occur through plasmid exchange or facilitated dissemination of transposons.

As a result of assessing the results of the antibiogram of the microbial strain Streptococcus pyogenes in antibiotic substances - Table 3, significance shows the highest degrees of sensitivity to antibiotic substances: cefeprim -28mm, followed by ofloxacin -26mm, ampicillin-24mm, cephalosporin -22mm etc.

According to the scientific aspects subsequently reported, the initiation of this research led to the interpretation of some aspects of the mechanisms of virulence in infections with the microbial strains Escherichia coli, Staphylococcus aureus and Streptococcus pyogenes noted in this study. Therefore, the additional virulence-determining bacterial toxins found in the noted strains are imported molecules. These include hemolysins, cytotoxins, proteins that bind various aggressin compounds.

Based on these considerations, we note that the bacterial strains studied that induce pathological inflammation in the form of abscesses, have evolved persisting longer in the animal body, without causing clinically obvious symptoms. These bacteria exist in a host-like relationship with commensal bacteria, and in some cases appear to protect the animal's body from colonization by other pathogenic strains.

In this context, from the presented analyzes we mention that the results obtained show that the antibiotic resistance of the strains involved is an important problem in the laboratory microbial pathology of abscesses at slaughtered pigs, which must be taken into account in the treatment of different abscesses by different origin of the species tested above for sensitivity and must be considered an issue that needs to be addressed in this context as well and commitments need to be made at national and international level.

CONCLUSIONS

1. The study confirms the dominance of antibiotic sensitivity spectra of microbial strains tested in the foreground by

Streptococcus pyogenes, followed by Staphylococccus aureus and E.coli.

2. Early recognition of abscess infections caused by microorganisms is the pursuit of prescribing appropriate treatment, depending on the sensitivity to antibiotics, preventing the acceleration of the manifested infection.

3. It is recommended to avoid the trauma, peripheral vascular diseases, ulcers and other pre-existing conditions and the timely administration of anti-inflammatory drugs in order to prevent pathologies of abscesses.

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IMPACT ON THE GROWTH PARAMETERS AND MICROBIAL POPULATIONS OF PROBIOTICS AND PREBIOTICS ON RABBITS RAISED IN THE HOUSEHOLD SYSTEM

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Abstract

The investigations and laboratory determinations were performed within the disciplines of Nutrition and Microbiology of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. The breeding of rabbits and the collection of samples took place in the own household from Sărata locality, Bistrița-Năsăud county. The aim of this study was to demonstrate the impact of the use of probiotics and prebiotics in feeding rabbits raised in the household system. For this experiment, 12 rabbits, common breed, 55 days old, raised in the household system were used. The basic feed ration was a commercial concentrated, ground feed in which the probiotic (Enteroferm® 35G), 0.15 g / kg feed, containing cultures of *Enterococcus faecium*, the prebiotic (Inulin FOS, ZENYTH), were incorporated, 25 g / kg fodder, powder obtained from chicory root (*Cichorium intybus*), which contains polysaccharides with a prebiotic role (inulin and fructo-oligosaccharide - FOS) and a synbiotic (mixture of the two), in the same doses. There were 4 groups of 3 individuals as follows: group number 1, control, group number 2, probiotic, group number 3, prebiotic and group number 4, synbiotic. In addition to the concentrated feed, good quality natural hay and water were added, both the feed and the water were administered ad libitum. In order to achieve the proposed goal, the amount of feed consumed by each group and the weight of each rabbit were determined weekly for 8 weeks. Based on these data, the final average weight of each group, the total feed consumption of each group, the weekly weight increase for each group, the average weekly and final feed conversion rate were calculated. Samples were also collected from the rectum, before and after treatment with probiotics and prebiotics, to determine their impact on the composition of the bacterial flora at this level.
The determinations performed yielded clearly superior results in favor of the experimental groups compared to the control group, highlighting group 2, consisting of rabbits treated with probiotics.

Key words: rabbits, probiotics, prebiotics, synbiotics

INTRODUCTION

Nowadays, rabbits are considered to be an important source of animal protein used in human consumption. They represent a unique segment in which they can be seen as livestock that is easy to manage. Also, the rabbits are highly prolific animals, with a short interval between generations. However, the costs implicated in the growth of the rabbits are very high (Adeyemo et al., 2013).

In the past, different additives were added to the daily food intake, in order to increase the food conversion rate and to obtain a good growth performance. From those additives, antibiotics were used in a high amount. However, in the growing context of antibioresistance, in January 2006, the European Commission forbiden the usage of antibiotics as growth promoters for animals, according to the Reglementation (EC) 1831/2003 (Reg EC 1821/2003). In this context, different alternatives were used with the same expected results for the growth performances, health status, or production performances, like antibiotics. In this category are included probiotics (Falcao-e-Cunha et al., 2007), prebiotics and sinbiotics. The term „probiotic" is defined as a mixture of live microorganisms that are able to provide a health benefit to the host when they are administered in adequate amounts (FAO/ WHO, 2002; Schmitz et Suchodolski, 2016). Prebiotics are composed of selective fermentative ingredients that are able to produce changes at the level of gastrointestinal microbiota (Gibson et al., 2010). The combination between the probiotics and prebiotics represents the synbiotics (Schmitz et Suchodolski, 2016). At present, probiotics are used on a large scale as growth promoters in animal husbandry. The advantage of those formulas is that the presence of residues in animal products used in human consumption is avoided, compared with antibiotics (Sherif, 2018). Moreover, it was demonstrated that probiotics are able to provide a

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health benefit to the rabbits, together with enhancing their growth performance (Bhatt et al., 2017). One of the main mechanisms of action of probiotics is represented by their capacity to improve the microbial intestinal balance of the host. Due to this ability, the microbiota present in the caecum of the rabbits is able to break down the cellulose, pectin, ammonia, urea, and the proteins from the small intestine (Sherif, 2018), improving the digestion and, at the same time, the growth performances.

The majority of the studies that researched the influence of different additives on the growth performances of the rabbits were performed on breeds designed for meat production, in an intensive breeding program, as specified for the industrial systems. The advantages of those systems are represented by the ability to have strict control over the feeding conditions and over the microclimate. However, in our country, the intensive breeding programs that use rabbits from meat breeds are neglected. Those systems are replaced by small household breeding systems. Unfortunately, the data regarding the influence of different additives (as probiotics, prebiotics, or synbiotics) on the rabbits' growth performances in this type of system are lacking. The aim of the present study was to evaluate the impact of probiotics, prebiotics, and synbiotics on the growth performances of the rabbits from household breeding systems.

MATERIAL AND METHOD

Study design

The present study was realized during 8 weeks with 10 day of adaptation. A total number of 12 rabbits, males, and females, aged between 55-60 days, clinically healthy were enrolled in the study. In the first 10 days of the study, the rabbits benefited from a period of accommodation. On day 11, rectal samples were collected (2 samples/group), in order to characterize the bacterial microflora resident at this level before the supplements administration. Also, the rabbits were weighed, and the initial mean weight was determined for each group. During the 8 weeks of the study, the rabbits were weighed once a week, and the mean weight for each group was calculated. At the same time, food consumption was monitored. At the end of the study, rectal samples were once again collected in order to compare the results with the initial ones. The weekly body weight gain for each group and the food conversion rate for each group were calculated. The study was approved by the Bioetic Commission of USAMV Cluj-Napoca with no. 254 /2021.

Biological material

Twelve rabbits, males, and females, mixed breed (Californian and English butterfly variety) were included in the study. The animals were randomly divided into 4 groups (3 animals / group). The animals were obtained immediately after weaning, at the age of 45-50 days, from a local breeder. Before the acquisition, a complete clinical examination was performed in order to select only the clinical healthy individuals.

Probiotic, prebiotic and synbiotic administration

The combined feed was represented by a commercial formula produced by S.C. PISANO GROUP S.R.L., presented as pellets, which were processed further by grinding, in order to allow the mixing between the food and the powder additives. The preparation of the mix between the food and the probiotic/prebiotic/synbiotic was realized manually, using a kitchen scale, a large capacity enameled vessel, and a hull for homogenization. After the homogenization, the ratio for each group was stored in plastic recipients, with sealing systems.

The used probiotic was composed of a single bacterial strain - *Enterococcus faecium* (Enteroferm® 35G). The product was administered in a quantity of 0,15 g for each kilogram of food (Kalma et al., 2016). The prebiotic was represented by chicory root (*Cichorium intybus*) (Inulin FOS by ZENYTH) and was administered in a quantity of 25 g for each kilogram of food (Juskiewicz, 2008). The symbiotic was represented by the combination between the probiotic and the prebiotic, in the same concentrations, and the administration followed the same protocol as for the other two groups

RESULTS AND DISCUSSIONS

Analysis of growth and development parameters

Mean body weight of the rabbits at the beginning of the study ranged from 1170 to 1257 g, at the end of the study reaching values between 2497 and 2858 g. Upon completion of the experiment, significantly greater body weights were recorded in the experimental groups, compared to control group. As can be seen in Table 1, the group 2, treated with probiotics, recorded the highest body weight gain (1643.26 g), followed by group number 4 (1601.65 g), treated with synbiotics, then group 3, treated with prebiotic and finally control group number 1.

In this context, we specify that the total body weight gain of group 2 was achieved with a total consumption of 23027 g of feed (table 2). In the case of group 4, the total body weight gain was achieved with a lower total feed consumption (22688 g) compared with second group. Analysis of the feed conversion rate evolution (table 3) shows fluctuations depending on the week, but the final average values indicate the best value for group 2 treated with probiotics (4.67 g of feed consumed / g body weight gain), followed by group 4 treated with symbiotics (4.72 g of feed consumed / g body weight gain).

Dynamics of microbial populations

Following the inoculation of samples collected before the start of treatment with probiotics, prebiotics and symbiotics, the appearance of bacterial colonies was polymorphic. Developed colonies were large, medium and small, of type S or R, white or yellow, morphological characters found in each of the 4 groups. Following the bacterioscopic examination, Gram + cocci were identified in 80% of the analysed microscopic preparations, being arranged in piles (in the form of clusters) and with an alveolar appearance in the microscopic field. These characters are specific to germs of the genus *Staphylococcus*. In 15% of the microscopic preparations were identified Gram + bacilli, unsporulated, morphological characters that can most likely include these germs in the genus *Bacillus*. In the remaining 5% of the preparations were identified coccobacilli, Gram-, lactose negative, represented by *Escherichia coli*.

Following the seeding of the samples collected at the end of the study, a different aspect of the colonies was observed. Medium and small, white and white to gray S and R type colonies developed. Colonies with yellow pigmentation were present, in small numbers, in groups 1 (control) and 3 (treated with prebiotics). The population of germs of the genus *Staphylococcus* was considerably reduced, they were identified in 15% of the preparations, in the largest proportion in the control group (group 1) and in smaller numbers in groups 2 (treated with probiotic) and 3 (treated with prebiotics). The control group presented a bacterial flora composed of 50% germs of the genus *Streptococcus*, cocci, Gram +, grouped in long chains, 30% bacteria of the genus *Staphylococcus* and 20% *Escherichia coli*. The rabbits in group 2, which were given the probiotic, showed a bacterial flora composed of 70% bacteria of the genus *Streptococcus*. From the rabbits in group 3, to which the prebiotic was administered, a bacterial flora composed of 80% *Escherichia coli* and 20% germs of the genus *Staphylococcus* was identified. From group number 4, treated with symbiotics, a bacterial flora composed of 90% *Escherichia coli* and 10% germs of the genus *Streptococcus* was identified.

In the context of what was observed in our study, Yamani et al. (1992) showed that the administration of probiotics to rabbits has beneficial effects on body weight and body weight gain. The same aspects are reported in 2004 by Amber et al., where after using *Lactobacilus acidophilus* supplements, the average daily gain of tested animals was greater with 9.6% compared to controls. At the same time, it has been observed that the administration of prebiotics and symbiotics significantly increases the body weight gain and the conversion rate of food (Ewuola et al., 2011).

The correlative analysis of the obtained data shows that the administration of probiotics, prebiotics and symbiotics modified the structure of the microbial population at the level of the rectum, with beneficial effects on the parameters of growth and development.

CONCLUSIONS

The results obtained indicate a favorable impact of probiotics, prebiotics and synbiotic on the growth indicators and on the microbial population from intestine of rabbits raised in the household system.

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Table 1

Mean weekly weight gain (WG) and total weight gain (TWG) of the 4 experimental groups (grams)

	Week 1	Week 2	Week3	Week 4	Week 5	Week 6	Week 7	Week 8	TWG
Group 1	136.89	142.95	161.64	159.44	158.34	162.91	186.92	217.68	1326.78
Group 2	176.64	187.60	218.99	208.95	238.06	211.97	188.39	212.67	1643.26
Group 3	131.36	156.10	198.60	191.57	201.81	200.86	200.98	196.86	1478.12
Group 4	159.33	167.08	184.55	187.54	202.47	205.00	240.17	255.51	1601.65

Table 2

Weekly and total feed consumption of each group (grams / group)

	Week 1	Week 2	Week3	Week 4	Week 5	Week 6	Week 7	Week 8	Total
Group 1	1825	2385	2781	3145	3261	3384	3578	3892	24251
Group 2	1909	2251	2887	2924	3177	3304	3167	3408	23027
Group 3	1814	2545	2906	3370	3629	3789	4002	4317	26372
Group 4	1810	2138	2428	2638	3044	3242	3521	3867	22688

Table 3

Feed conversion rate (FCR) in the experimantal groups for 8 weeks and total period (TFR)

	Week 1	Week 2	Week3	Week 4	Week 5	Week 6	Week 7	Week 8	TFR
Group 1	4.43	5.66	5.81	6.50	6.84	6.94	6.39	5.99	6.09
Group 2	3.60	4.03	4.42	4.65	4.43	5.18	5.60	5.33	4.67
Group 3	4.66	4.79	4.82	5.02	5.23	5.51	5.27	5.77	5.95
Group 4	3.78	4.27	4.39	4.69	5.01	5.28	4.93	5.09	4.72

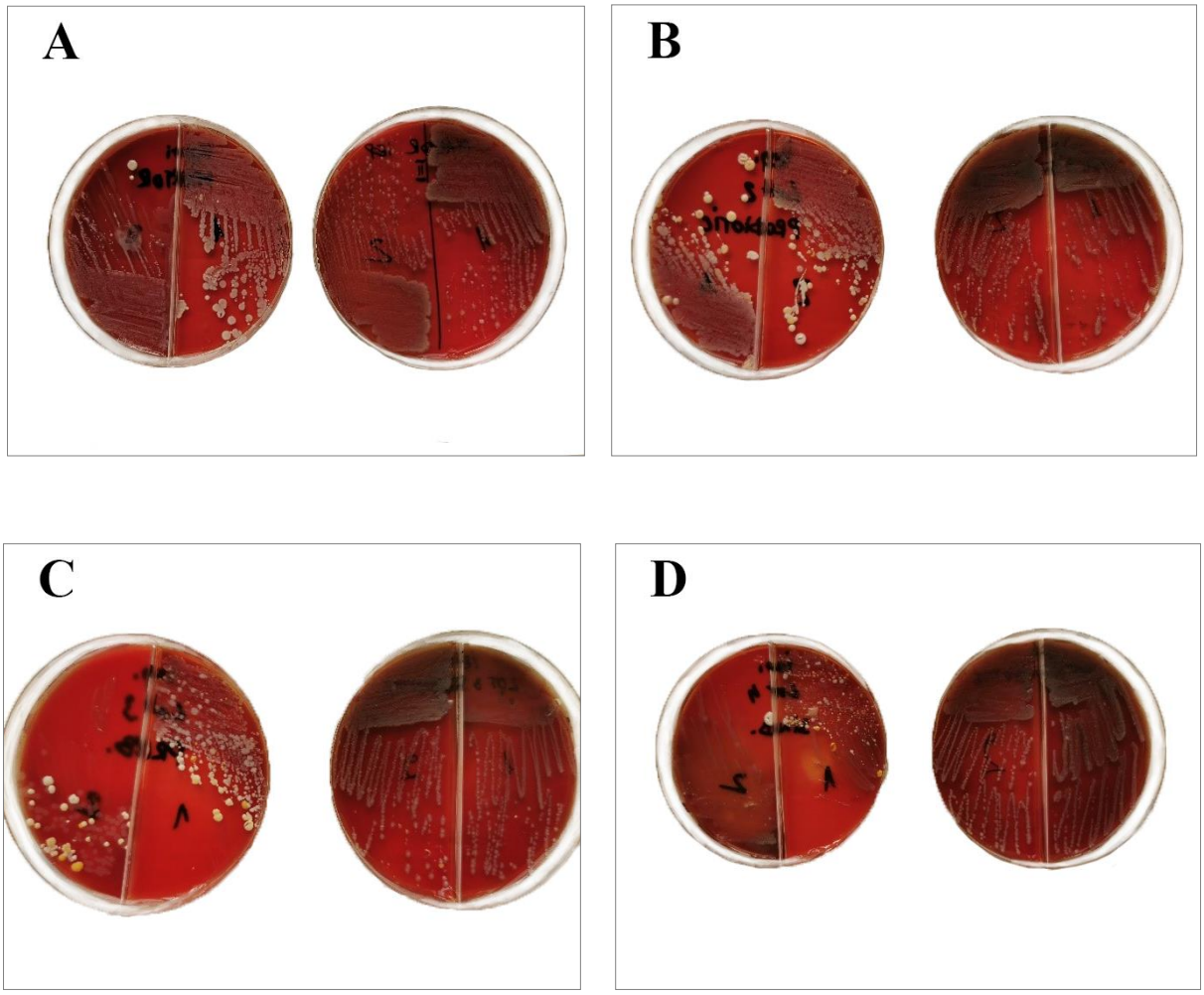

Figure 1 The morphological aspect, ante-therapeutic (left) and post-therapeutic (right), of the bacterial colonies: A. Lot 1 (control); B. Lot 2 (probiotic); C. Lot 3 (prebiotic); D. Lot 4 (synbiotic).

ASSESSMENT OF DIGESTIBILITY AND FECAL SCORE OF RAW MEAT-BASED DIET (B.A.R.F.) IN DOG FEEDING

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Abstract

The aim of this study was to determine digestibility in the B.A.R.F (Biologically Appropriate Raw Food) diet. The study was performed on a number of 5 dogs, from which fecal samples and a sample of their ration were collected over a period of 5 days. Some of the reasons for owners to choose this diet were: longer and healthier life, lack of dental tartar, no smell of the oral cavity, the appearance of fur, solving dermatological problems, lower fecal volume and lower defecation frequency, more energy and less expensive. One of the benefits of the BARF diet is the reduced volume of feces, due to optimal digestion and high absorption. Once one makes the switch to the BARF diet one will notice that their pet will defecate less and its consistency will be compact, dark in color. Exceptions occur when the diet is not properly balanced. Following the determination of the digestibility of the BARF diet in dogs, we obtained high values especially for protein (96.55%) and fat (99%). The value obtained for mineral substances of organic origin was also high (54%). We consider that the high digestibility is due to a very good adaptation of the digestive tract to the natural ingredients used in the diet.

Key words: B.A.R.F. diet, dog, digestibility, fecal, score.

INTRODUCTION

B.A.R.F. (Biologically Appropriate Raw Food) is a diet based on raw foods. We are talking about a diet for dogs and cats that has evolved over a million years of genetic adaptation. The "BARF Program" was first introduced to the world in 1993 through the first book, "Give Your Dog a Bone," written by Ian Billinghurst, a graduate veterinarian at the University of Sydney, Australia, in 1976. The diet was accepted easily by animal owners but also by kennel breeders. In recent years it has become widespread among users of industrial feed. Owners have become increasingly concerned about the health of their animals, encountering more and more degenerative diseases have become alarmed and have successfully adopted the "program". And because this diet is based on the nutrition that our animals have had for millions of years, for them it is not a novelty, it is not a radical change. In fact, it is a return to a proper organic food system, abandoned 60-70 years ago, when industrial food grew. Why is the evolutionary diet so "magical"? Simple ... Because as any apparatus that works properly if it is supplied with fuel or spare parts recommended by the manufacturer, so do animals are adapted to specific feedstuff. Therefore they need an "evolutionary" diet because it is the one recommended by the "producer", ie their digestive system, their body, their nature, namely that of carnivore. It is a diet able to improving health, longevity and productive capacity. (Billinghurst I, 2001) 10-15% of the dog's diet should consist, according to BARF diet, of entrails or internal organs such as liver, kidneys, heart, brain, tongue and lungs. They must be fresh and raw. It must come from a reliable source and not contain parasites. Raw organs are a valuable source of nutrients, including water, protein, essential fats, vitamins and enzymes. (Reinerth S, 2015). The most important organs are the heart and liver. The liver should not exceed 5% of the total organs (very important) being an organ rich in vitamin A, we can reach a excess of vitamin A. Ex: if a dog eats 600g a day, it should have a meal of fish in the amount of 480g, which will be joined by vegetables in the amount of 120g or sweet potatoes boiled in the same amount. A full 100% meal with fish can be administered once a week. It can be administered whole, but it needs to be frozen before, at least 96 hours, to kill most parasites, the most common being the ones from *Genus Anysakis* (Billinghurst, 2017; Mihaiu, 2015).

Transition to the B.A.R.F. starts with a day of fasting before introducing the new diet, to give

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the body time to eliminate the toxins accumulated from the previous diet. Another reason for opting for fasting day would be to speed up the increase in stomach acidity, acidity that helps digest raw foods. A protein test is performed (a single protein for 2-3 days), in the first days, to detect possible intolerances to a certain protein; intolerance that we will notice through diarrhea or vomiting. In this case, we will eliminate the protein from the diet. Once the proteins have been tested, small amounts of fleshy, soft bones are introduced initially. If the animal reacts well, only then will we be able to integrate the organs (for a week), to which the belly will be added later (the next week) and finally the vegetables or fruits one type each.

Each dog has its own requirements based on age, activity, weight and other personal factors (Case, 2010). For a healthy dog, with a normal physical activity, which has reached maturity, 3% of its ideal weight will be calculated; that is, to the maximum shape. No organs are given to a beginner dog in BARF (if the diet started only a few days ago) because they have a slightly laxative effect. The belly in the BARF diet has a probiotic role, being rich in bacteria and enzymes that help digestion. It is given in the amount of 10% of the total daily food of the dog. You can give a belly of lamb, beef or sheep. If there is no green belly, its amount will be replaced with lean meat, and the role will be assumed by kefir (one tablespoon for every 10 kg of dog weight).

MATERIAL AND METHOD

The study had the following objectives: determining the digestibility of dogs fed the B.A.R.F diet and assessing the fecal score. The biological material was represented by 5 dogs, 3 males and 2 females, aged between 1 and 6 years. BARF diets, administered to the dogs studied over a 5 day collection period (control), consisted of a wide range of foods (*table 1*). The raw chemical composition of the diets was determined using the Weende methodology. The apparent digestibility coefficient (ADC) for dry matter, crude protein, crude fat, crude ash and nitrogen free extract (NFE) were calculated (Macri, 2014).

$$\text{ADC \%} = \frac{\text{intake-excretion}}{\text{intake}} \text{ x 100}$$

The coproparasitological examination was performed to monitor the presence of oocysts, protozoan cysts, oncospheres and trematode eggs. The method used for parasitological examination was flotation. After examining the preparations under a microscope, they came out negative, digestibility trial not being affected by parasitic influence. The fecal score was also determined, observing: feces with undigested bone fragments, feces too hard, cementitious or crumbly, feces too soft, gelatinous feces (with mucus). Fecal score was assesd according to Purina ProPlan® Veterinary Diets diagram of the fecal score and characteristics of the nutritional management of GI Canine Health. (www. proplanveterinarydiets.ca). Assessment of dog appearance and behavior: fur, breath smell, teeth, alertness was done using a questionnaire that the owners fill at the end of our study.

RESULTS AND DISCUSSIONS

The analysis of the BARF diet administration, we mention that the owners observed a positive effect on their health. In this context, we mention the improvement of the appearance of the fur, the disappearance of bad breath and a cleaner appearance of the teeth. It was also observed a reduction in the amount of feces, which can be correlated with increased levels of digestibility. Effects on the nervous system were observed, manifested by increased alertness and agility. The results of the fecal score and some characteristics for each dog are presented in table 2. In Table 3, the apparent digestibility coefficient (ADC) is presented, observing higher values of in protein and fat, compared to commercial food. In our study for dogs that followed the BARF diet feedstuffs presented values between 90.6% - 98.51% for crude protein and 98.63% - 99, 61 % for crude fat (*table 3*). ADC for dry matter was between 85.68 % - 91.18%. Daumas studied the digestibility of different commercial diets in dogs. For apparent digestibility, the range of crude protein and crude fat values was 66.9 % - 84.4% DM 70.4 % - 82.5% crude protein and approximately 95% for crude fat. In another study, Hagen-Plantinga et al. (2014) determined the apparent digestibility of 89 % crude protein and 94-97 %crude fat and in dog that were given commercial diet 76% -89% crude protein and 94%-97% crude fat. The average values obtained for the apparent digestibility of dry matter, crude fat, and nitrogen are 77%, 94% and 78%. Meyer et al (1999) presented similar results for digestibility of commercial diets. Apparent digestibility of the organic matter was 88.9 % FOR dry diet, without detectable breed differences. Up to 88.2% was the crude protein digestibility and (84.9–89.4%) for the canned food. Though a high digestibility has shown the crude fat (93.8 and 96.4%) similar in all studied breeds. It should be noted that the study was limited. Dogs differ in breed, age, weight. Both the breed and the difference between environmental conditions (type

of diet, daily effort, his habits, and the owner) play a significant role in the results. According to literature, the collection of feces is done over period of five days, obtaining a representative sample. A shorter collection period will not be significant. The mean frequency of defecation ranged between 1,2 and 1.4 during our study which can be considered normal. Every animal had a minimum of 1 stool per day so no constipation was reported. In the Meyer at al (1999) investigation the frequency of defecation was between 1 and even 2.7 for canned diets.

Feces score was appreciated to be between 1 and 2, with an average of 1.2.

Fecal score evaluated by Felix et al. (2010) in a 1 to 5 scale as: 1 = very soft feces to 5 = shaped, dry, and hard feces according to SÁ-FORTES (2005) was higher for dogs fed a diet supplemented with Bacillus subtilis (C-3102) (3.4 vs. 3.0) than dogs fed with the control diet.

CONCLUSIONS

The BARF diet showed a high digestibility due to the better adaptation of the digestive tract to this type of food. Average fecal score was 1.2.

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Table 1

Combinations of feedtuffs used during the study

		Dog 1 – 600g	**Dog 2 – 690g**	**Dog 3 – 540g**	**Dog 4 – 510g**	**Dog 5– 540g**
Day 1	Meat with bone- 270g	Turkey	Chicken	Duck	Rabbit	Chicken
	Meat without bone - 150g	Rabbit	Cattle	Zucchini	Chicken	Turkey and duck
	Legumes / Fruits – 60g	Apple	Carrot	Zucchini	Zucchini	Apple, carrot and zucchini
	Organs – 60g	Cattle and duck	Rabbit	Cattle and duck	Cattle and duck	Cattle and lamb
	Stomach – 60g	Cattle stomach	Cattle stomach	-	-	Shrimp
Day 2	Meat with bone - 270g	Rabbit	Duck	Turkey	Salmon	Chicken
	Meat without bone - 150g	Duck	Chicken	Cattle	Duck	Turkey and duck
	Legumes / Fruits – 60g	Carrot and cucumber	Cucumber	Pear	Carrot	Spinach and parsnip
	Organs – 60g	Cattle and turkey	Cattle and chicken	Cattle and chicken	Cattle and chicken	Chicken and lamb
	Stomach – 60g	Cattle stomach	Cattle stomach	-	-	-
Day 3	Meat with bone - 270g	Chicken	Rabbit	Baby Herring	Duck	Chicken
	Meat without bone - 150g	Sheep	Sheep		Cattle	Chicken
	Legumes / Fruits – 60g	Pear	Apple		Kiwi and pear	Pear
	Organs– 60g	Rabbit	Cattel and turkey		Cattle and duck	Cattle and lamb
	Stomach – 60g	Cattle stomach	Cattle stomach		-	-
Day 4	Meat with bone - 270g	Salmon	Hake fish	Chicken	Chicken	Duck
	Meat without bone - 150g	Turkey		Duck	Turkey	Duck and chicken
	Legumes / Fruits – 60g	Spinach and zucchini		Apple	Cucumber	Arugula, cucumber and parsley
	Organs – 60g	Cattle and sheep		Cattle and turkey	Cattle and duck	Chicken and lamb
	Stomach – 60g	Cattle stomach		-	-	-
Day 5	Meat with bone - 270g	Duck	Turkey	Rabbit	Chicken	Duck
	Meat without bone - 150g	Cattle	Cattle	Cattle	Cattle	Chicken
	Legumes / Fruits – 60g	Banana and kiwi	Spinach and cucumber	Zucchini	Apple	Beet and zucchini
	Organs – 60g	Cattle and chicken	Cattle and duck	Cattle and chicken	Cattle and chicken	Cattle and lamb
	Stomach – 60g	Cattle stomach	Cattle stomach	-	-	-

Table 2

The results of the fecal score and some characteristics for each dog

Dog 1 (male)	**Day1**	**Day 2**	**Day3**	**Day 4**	**Day 5**
Fecal score	1	1	2	1	1
Fecal volume	Small	Small	Small	Small	Small
FOD	1 / day	1 / day	2 / day	2 / day	1 / day
Dog 2 (female)	**Day1**	**Day 2**	**Day3**	**Day 4**	**Day 5**
Fecal score	1	1	1	1	1
Fecal volume	Small	Small	Small	Small	Small
FOD	1 / day	2 / day	1 / day	2 / day	1 / day
Dog 3 (female)	**Day1**	**Day 2**	**Day3**	**Day 4**	**Day 5**
Fecal score	1	1	1	2	1
Fecal volume	Small	Small	Small	Small	Small
FOD	1 / day	1 / day	1 / day	1 / day	2 / day
Dog 4 (female)	**Day1**	**Day 2**	**Day3**	**Day 4**	**Day 5**
Fecal score	2	2	1	1	1
Fecal volume	Small	Small	Small	Small	Small
FOD	1 / day	1 / day	1 / day	2 / day	1 / day
Dog 5 (male)	**Day1**	**Day 2**	**Day3**	**Day 4**	**Day 5**
Fecal score	1	1	2	1	1
Fecal volume	Small	Small	Small	Small	Small
FOD	1 / day	1 / day	2 / day	2 / day	1 / day

FOD= frequency of defecation

Table 3

Apparent digestive coficients in the BARF diet

Dog	***Dry matter***	***Crude protein***	***Crude fat***	***Crude ash***	***NFE***
1	85,68	90,60	99,61	49,01	69,36
2	89,42	97,80	99,14	63,01	52,29
3	88,73	98,12	98,63	68,06	48,87
4	86,35	97,74	99,51	48,88	41,70
5	91,18	98,51	98,98	66,97	58,19

THE IMPORTANCE OF COMPUTERIZED RATIONS AND THEIR IMPACT IN A DAIRY COW FARM IN THE BOTOȘANI REGION

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Abstract

The nutrition of dairy cows plays a very important role especially in terms of farm economy, efficiency and health of dairy herds. Unfortunately, in many farms this aspect is treated superficially and economic efficiency is not maximized in order to obtain the highest milk yields and efficient use of feed. In view of the above, we have chosen this topic in order to achieve the most efficient computerized ration in the herd under study. In this case we followed, over a period of 10 weeks, the impact of the computerized ration and the control ration on milk production both in terms of quantity and quantity, and on feedstuff usage optimization. Another aspect followed was the economics of forage and finance in the farm. The study also relied on chemical analysis of the feed to determine the quality of the feed. For this we determined the chemical composition of the feed by looking at the amount of protein, cellulose, fat, then followed the actual implementation of the ration and at the end all the data was collected to show the efficiency of this study. There were two experimental batches, grouped by weight and productivity, also the control batch was followed. During the 10 weeks we noticed that the feed quantity was lower and the milk yields remained relatively constant with little impact on milc chemical composition. In this way we have successfully highlighted the importance of implementing such specialized computer software in a dairy farm where rations are made without a scientific basis.

Key words: dairy cows, ration, economy

INTRODUCTION

Nowadays, a new approach to animal husbandry is being pursued, especially dairy cows. In this sense, many years ago, the political situation imposed an increase in cattle in the collective system, in farms of impressive size but after de-collectivization disappeared over time. Small family farms reemerged and those of very large size being in smaller numbers. The current trend is changing, in the sense that family farms tend to expand and farms with a number of 2-3 cattle are beginning to disappear. That is why is consider that a more efficient way comes through mechanization of physical work because the labor force is deficient, and at the same time another important thing is rational feeding of dairy cows. This goal is to avoid wasting feed, increase production and milk quality. One last thing pursued for an efficient development is related to the fact that a genetic improvement of the breeds is also tried for productivity as advantageous as possible for the farmers. (Gutierrez-Reinoso et al., 2021) In animal husbandry the aim is to obtain productions with lowest possible costs. In this sense, it must be considered animal growth in an manner that ensures a microclimate as favorable as possible so that energy losses to be as small as possible. To achieve this, over the years cow farms have adopted various breeding systems. Today, in some high-tech farms, human intervention is minimal and the need for staff is much lower, which helps to maximize profits. Also, today's genetics helps farmers to obtain significant milk production, in some farms the average being over 40 l. per animal. (Acatincăi S., 2004) This advantage also comes with a major disadvantage related to the fact that these animals are extremely dependent on the environmental conditions and the ration administered and any major change leads immediately to significant losses. The maintenance systems are diverse, but they are divided into 3 categories, namely: maintenance in the stable system, maintenance in the summer camp system, maintenance in the mixed system. (Paşca et al., 2007)

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MATERIAL AND METHOD

The purpose of this paper is to assess the impact of computerized rations on milk characteristics and quantity and also on farm efficiency. The quality of feedstuffs used in the feeding of dairy cows used for both production and reproduction was observed. Samples were taken to highlight the quality of rations used on the farm and to assess their chemical composition. Thus, the organoleptic examination was performed, the raw chemical composition was determined by Weende Method and the rations was analyzed used by means of a computer program were assessed. (Hybrimin Flutter 5.1 ®).

The number of cattle on the farm is 44, of which 22 are adult dairy cows, 16 are young females and 6 are males. After determining the chemical composition of the feed that enters the ration of the cows on the farm, the animals were subdivided according to their weight and the amount of milk produced daily. Of the 17 lactating cows at the time of the experiment, we chose a number of 16, of which 8 were selected in the control group and another 8 in the group subjected to computerized ration. The group of cows subjected to computerized ration was further divided into 2 other groups of 4 in order to have homogeneous groups. We chose to carry out this process because there was a much too big difference between the least productive and the most productive cattle and the ration in this way would not have been balanced and correct. The computerized rations were implemented for 10 weeks, investigating at the end of each week the quantity of milk. The quality was appreciated in the first week, the fifth and also in the last week using apparatus Lactoscan SAP®. To determine the optimal ration, we used the computer program Hybrimin Futter 5.1®. In order to adapt the ration as accurate as possible for each category of animals, we made two experimental groups of animals. Thus, the first experimental group had an initial production average of 14 liters and a body weight of 550 kilograms, and experimental group 2 initially had an average of 20 liters and the average body weight was 570 kilograms. On the other hand, the control group has an average milk production of 14 liters and the average body weight is 550 kilograms. The number of animals per group was 4 with similar body weights and productive performance. The computer software mentioned above, in addition to the structure of the groups of animals and the establishment of milk production, the types of utilized feedstuffs was necessary. The investigated feedstuffs ware represented by: maize silage, natural hay, maize grains, wheat grains, alfalfa hay, combined fodder, sunflower meal. Ration for the tree experimental groups are presented in table 1.

RESULTS AND DISCUSSIONS

Following the laboratory analyzes for the 8 feed samples taken from the farm, different nutritional values were found depending on the type of feed. Juicy-silage (maize silage), coarse fodder (alfalfa hay, hay from natural pastures), combined concentrates, but also the ingredients from which they were obtained were analyzed. The quality of the feed was assessed in terms of its raw chemical composition. From this point of view, we appreciated the fact that the fodder had values of humidity within normal limits, except for maize. Their composition in organic substances such as: protein, fat, cellulose, ash and non-nitrogenous extractive substances is close to values or are in the range of normality of those specified in the international literature. (Preston, 2013) (Souvant et al., 2004)

The humidity measured in the case of maize grains (18.36%) was above the limits that have been cited in the literature. This aspect is quite important because, in agriculture, the humidity of cereals plays an important role due to the desideratum related to storage. In this sense, a high moisture content of cereals can lead to significant losses, especially in the case of grain depots where there is no dryer upon receipt. The danger of harvesting and storing such fodder occurs when they are stored and after a period there is a great possibility of developing the phenomenon of mold. Thus, the fodder affected by this phenomenon ends up becoming an economic loss for farms, in case of neglect of this aspect reaching significant losses. From the point of view of farms raising cows for milk production, the danger of using concentrates with high humidity is significant, because they can develop mycotoxins, which are excreted in the milk secretions of animals. Mycotoxins are considered according to recent studies with carcinogenic potential and their detection in milk delivered to processing plants has very serious consequences on the responsible farm. (Sultana and Hanif, 2009)

According to a study, in some farms milk production is higher than other farms where chemical structure of feedstuffs is ignored. In other words, the milk production varied in proportion to the amount of protein in the analyzed feed. (Assaminew and Ashenafi, 2015). After obtaining the computerized ration, the administration of the ration for the two experimental groups started on the farm.

In the first week after administering the optimized ration, both experimental groups

underwent quantitative changes in milk (*table 2*), so that the average of both groups decreased by about 1 liter and in the third week the decrease continued in the case of the second group with more1 liter,. Also, in the third week, group 1 reached a minimum of milk production (1.5 liters) compared to the average initial production. In the following weeks, milk production began to increase slightly to values close to the initial ones, reaching similar values in weeks 9 and 10 and the average in both groups being 0.5 liters lower compared with the initial measurement. Milk production was affected by the implementation of the optimized ration, but the decrease in production was not significant. Another fact is that, in the last 3 weeks, for the experimental groups the milk production was constant. Studies on this aspect indicate that optimized rations help to achieve milk production without significant fluctuations (Coșman, 2017).

During the 10 weeks of the experiment, an analysis of the main constituents of milk was performed (*table 3*). The chemical structure of the milk was affected in the case of group 1 and group 2. These changes are more relevant in the case of fat percentage and amount of protein. The decrease in the first 5 weeks was 0.2 percent for both groups in the case of fat, respectively 0.1 grams for group 1 and 0.3 grams, values that refer to the amount of protein. However, these decreases were not found in the last determination in week 10, the values being very close to the initial ones. The initial decrease can be attributed to the fact that there has been a change in the ration and its content is no longer with an excess of fat and protein. However, recent studies by Cavallini et al (2018) shows that, for dairy cows with optimized ration the fluctuations of milk production and quality are not significant and on another hand, in those that are not intervened to optimize the ration, the fluctuations were significant.

Also, in addition to the nutritional factor, qualitative and quantitative fluctuations in milk production may also be due to hormonal causes. According to the study conducted by Lopez et al. which found that cows with an average production of 30 liters per day can have a decrease of 5-10 liters due to the estrous period. The administration of excess feed is a common problem, because dairy cows reach a maximum of productivity with a certain amount of nutrients, but above this threshold the administration is in vain and leads only to losses. A one-year study of dairy farms in Kosovo shows that their production is optimal, constant, but most do not calculate the necessary nutrients related to the weight and productivity of each animal (Shkodra, 2020). During the 10 weeks, there was a significant change in consumption in the sense that for the experimental groups, there was a decrease in ingested feed. However, this decrease was not negatively affected on milk production because, as mentioned above, the quantity and quality of milk fluctuated but returned to values close to the initial ones. Analyzing the feed economy achieved we can see that there are quite large quantities and such surplus feed can be capitalized on in several ways. First of all, the farmer can consider an increase in the number of animals or the feed that is in surplus can be capitalized by sale. For both cases, the farmer's profit is higher, compared to the case where he continues with the ration from the control group where he manages surplus fodder that is not capitalized efficiently by the animal.

The calculations refer to the 10 weeks in which the computerized ration was implemented, and these are based on the consideration of only one animal in the group. In other words, in the case of group 1, for a single animal, the economy from the financial point of view, during 10 weeks was of 440 lei and in the case of the second lot, the amount was represented by 381 lei. Extrapolating the financial result, for one year we come to the conclusion that the economy on the farm can reach an amount of about 2,500 lei per single animal. For a medium capacity farm, where there is a herd of 20 cows, we can reach the amount of 50,000 lei. According to a study by Dean et al. In the United States, in 1972, for a year, they managed to implement a computerized ration to minimize overfeeding. They noticed stabilization of the average milk production, and in the end, the financial result showed that for each animal in the group, the saved feed was worth $ 1.6 per day.

CONCLUSIONS

The feed, from an organoleptic and chemical point of view, was classified as good and very good quality. Milk chemical composition was similar to control after 10 weeks. Adapting the ration of cows to requirements reduced the quantity of feedstuffs and so the cost of animal feeding. Computer designed rations help to achieve significant feed savings.

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Table 1

Ration structure used in the experimental groups

Name of ingredient	*DM (g / kg)*	*Group 1*		*Group 2*		*Control group*	
		Total DM (kg)	Quant. (kg)	Total DM (kg)	Quant. (kg)	Total DM (kg)	Quant. (kg)
Natural hay, older	860	3.338	3.882	3.010	3.500	6.880	8.000
Alfalfa hay	860	4.674	5.434	5.354	6.225	8.600	10.000
Maize silage	250	3.000	12.000	2.643	10.574	3.000	12.000
Sun flower cake	899	0.483	0.538	0.774	0.861	0.450	0.500
Maize grains	870	1.740	2.000	3.480	4.000	3.045	3.500
Wheat grains	870	0.384	0.400	0.950	1.092	0.435	0.500
Oat grains	870	0.522	0.600	0.261	0.300	0.870	1.000
Sodium cloride	970	0.021	0.021	0.028	0.029	0.049	0.050

Table 2

Average milk production (l/animal/day) during experimental period with optimized and control rations

Date	*Week 1*	*Week*	*Week*	*Week*	*Week*	*Week*	*Week*	*Week*	*Week*
Group 1	12.25	11.22	10.57	10.72	10.92	11.22	11.55	11.62	11.67
Group 2	17.12	16.05	15.30	15.45	15.62	16.05	16.55	16.47	16.50
Control	14.00	15.30	14.80	16.00	16.20	15.60	14.20	14.70	15.40

Table 3

Milk analyses in first, fifth and tenth week of study

	Milk analyze – week 1							
	Somatic cells x1000	**Fat (%)**	**Protein (%)**	**Lactose (%)**	**Urea (mg/100g)**	**Casein (g/l)**	**Density (g/l)**	**pH**
Group 1	120	4.26	3.81	4.82	31	29.3	1032	6.27
Group 2	168	4.22	3.83	4.89	38	28.5	1031	6.32
Control	118	4.24	3.76	4.77	35	29.1	1029	6.22
	Milk analyze – week 5							
Group 1	120	3.98	3.71	4.83	34	28.3	1033	6.17
Group 2	168	4.11	3.53	4.81	32	28.9	1030	6.22
Control	118	4.27	3.96	4.71	33	29.5	1027	6.12
	Milk analyze – week 10							
Group 1	120	4.19	3.73	4.79	33	29.3	1030	6.29
Group 2	168	4.17	3.63	4.72	37	28.5	1031	6.20
Control	118	4.2	3.46	4.82	34	29.1	1031	6.25

ASSESSMENT OF FEED QUALITY AND CONTAMINATION OF FEEDSTUFFS WITH TOTAL AFLATOXIN AND ZEARALENONE IN A DAIRY COW FARM IN BISTRIȚA-NĂSĂUD COUNTY

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Abstract

This study was conducted in a dairy cattle farm in Bistrița-Năsăud County. Assessments were made over the fodder used to feed dairy cows and mycotoxic load. Feed samples were collected two times, first in the fall of 2019 and again in the fall of 2020 from different places from the same batch and they were conditioned shortly after harvest by drying and grinding. A total of 8 samples were collected from the farm and they represent the total of all feed used in the livestock feed in that farm. All of the forages were organoleptically analyzed, we determined the chemical composition; dry matter, crude protein composition, ether extract, crude cellulose, crude ash and nitrogen free extract. Zearalenone and Total Aflatoxins were also determined from each of the samples using RIDASCREEN® test, which are an competitive enzyme-linked immunosorbent assays. Zearalenone was detected in all the samples analyzed with values between 43.83 and 1054.03 µg/kg in 2019 and in 2020 with values between 103.45 and 1818.23 µg/kg. 75% of the samples analyzed in 2019, 50% of the samples analyzed in 2020 exceeding the maximum permissible limit in the European Union (EU). Total Aflatoxins were detected in all the samples analyzed, with values between 0.361 and 2.35 µg/kg, without exceeding the maximum permissible limit in EU.

Key words: bovine, gross chemical composition, zearalenone, aflatoxins

INTRODUCTION

Animal nutrition is a very important aspect in animal husbandry, even if this aspect is not taken seriously by Romanian farmers. Most diseases are caused by poor quality feed, poorly designed rations by empiricists and mold-contaminated feed that leads to mycotoxin poisoning that worsens the health of animals and leads to decreased productivity, especially combined with poor feeding. The quantitative and qualitative control of the feeds is done through a series of laboratory analyzes, intended exactly to detect their non-conformity. An important role that these laboratories play is to test the presence of toxins in feed and to provide the nutritionist with the necessary data on the nutritional value of feed (Tisch, 2005). Among the chemicals involved in the occurrence of biological and economic problems in animals, we mention mycotoxins, produced by certain species of fungi such as: *Aspergillus, Fusarium, Penicillum, Trichobacterium*, etc. Not all mycotoxins are important for human food safety, but aflatoxins, ochratoxins, fumonisins, patulin, ergotoxin, and trichothecine are exceptions (Diaz, 2005). Mycotoxins are toxic substances produced by fungi (molds) that grow on field crops or on stored ones. Of the several thousand species of mold that can grow on feed, only a few produce mycotoxins. Even if over 400 mycotoxins are identified chemically, the biological or veterinary impact is known to only a few (Seglar, 2017). Several studies have identified the action of mycotoxins in ruminants. Dairy cows suffering from mycotoxicosis have been associated with decreased milk production and failure to respond to therapies or dietary changes. Symptoms are usually not specific and may include: reduced food intake, refusal of food, poor body condition and reproductive problems. Field investigations were associated with abomasum displacement, ketosis, placental retention, metritis, mastitis, and fatty liver (Selgar, 2017)

MATERIAL AND METHOD

The study was conducted between 2019 and 2020, on a farm in Bistrița-Năsăud County and in the discipline of Animal Nutrition, within the Faculty of Veterinary Medicine Cluj-Napoca. The farm is located in Orheiu Bistriței, Bistrița-Năsăud County. It consists of 2 halls that house 400 head of cattle, of which 150 dairy cows and the rest cows in

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breastfeeding, pregnant youth, youth for preparation for calving and calves.

The analyzes were performed on a number of 8 samples of fodder taken from the farm located in Bistrița-Năsăud county from Orheiu Bistriței, 4 fodder samples from 2019 (sample 1- maize silage, sample 2 – maize and grass silage, sample 3 - combined feed for cows, sample 4 – natural hay) and 4 samples from 2020 of the same feedstuffs . Partial samples were taken from several lots, 0.1 kg each. After homogenization, average laboratory samples (maximum 1 kg mass) were extracted by square method. The organoleptic examination of the fodder on the farm was performed; the analysis of the raw chemical composition of the 8 fodder samples was determined by the Weende method and the mycotoxicological examination, determining the level of Zearalenone and total Aflatoxins in the examined samples. To perform the mycotoxicological examination we used the RIDASCREEN® Zearalenone test, which is based on competitive enzyme-linked immunosorbent assays to determine the quantitative determination of zearalenone in cereals, feed, beer, serum and urine. For the quantitative analysis of total aflatoxins, 4 feed samples were used. Laboratory analyzes were performed with the RIDASCREEN®FAST Aflatoxin ELISA competitive immune-enzymatic ELISA test for the quantitative determination of aflatoxins in feeds. The interpretation of the results was performed according to the EC Regulations No. 1881/2006 and No. 1126/2007 regarding the limits of mycotoxins in fodder and food. The statistical analysis was performed using Microsoft Excel.

RESULTS AND DISCUSSIONS

The results of the organoleptic examination of fodder used in cow feed in 2019 ranged from good to very good quality. The sample of maize and grass silage showed a greenish-yellow color, with a pleasant smell, it did not show impurities, so the assessment was as having a good quality. The hay sample was appreciated as a good quality, presenting a green color, a pleasant smell and without impurities. The corn silage did not show any impurities, had a pleasant smell, a yellow color and was considered of good quality. Combined pelleted fodder (complementary feed) is the only one that was considered of a very good quality, with a pleasant smell, it did not present impurities and the color was the specific one, yellow-brown. Regarding the results of the chemical composition of the 2019 feed samples, there is a good quality of natural hay, with dry matter values of over 87%, the protein having slightly higher than average values of about 8%, lower values are observed only in the case of crude cellulose. Regarding the values of complementary compound feed, it has a good value in terms of energy and nitrogenous substances, with a value of crude protein of over 24%. The value of the crude protein from the corn silage, compared to the dry matter, has lower values than its average in these varieties of fodder, namely 5.17% compared to an average value of approximately 8%. For the determination of Zearalenone, samples were collected from each batch of fodder from 2019. Zearalenone was detected in all samples examined, 3 of the 4 exceeding the maximum values allowed in the feed of dairy cows. Zearalenone values ranged from 41.83 µg / kg to 1054.03 µg / kg. The contamination is of moderate to high intensity, the feed with the highest concentration of Zearalenone being the corn silage with 1054.03 µg / kg, and the lowest concentration is in the complementary feed with 41.83 µg / kg. Of the 4 samples analyzed, 3 exceed the maximum allowed value of 500 µg / kg in cow feed. The results show that there are problems in the processing and storage of feed and this is a risk factor in terms of animal health, most often affecting the reproductive system. Given the fact that we have high concentrations of Zearalenone and after discussions with the farmer, it was concluded that there are problems caused by this mycotoxin. The farmer reported that he had problems specific to zearalenone intoxication: decreased milk production, infertility, abortions and embryonic resorption. A study on mycotoxin poisoning in Africa shows that due to the lack of awareness of the danger of feed containing mycotoxins, animals are still fed with this feed.

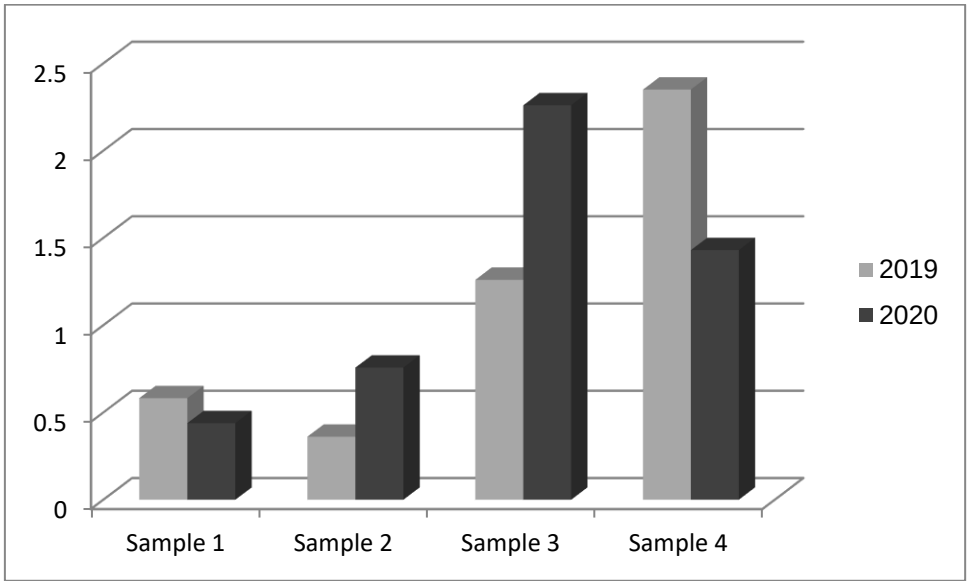

Figure 1 Total aflatoxin in the investigated samples in the years 2019 and 2020

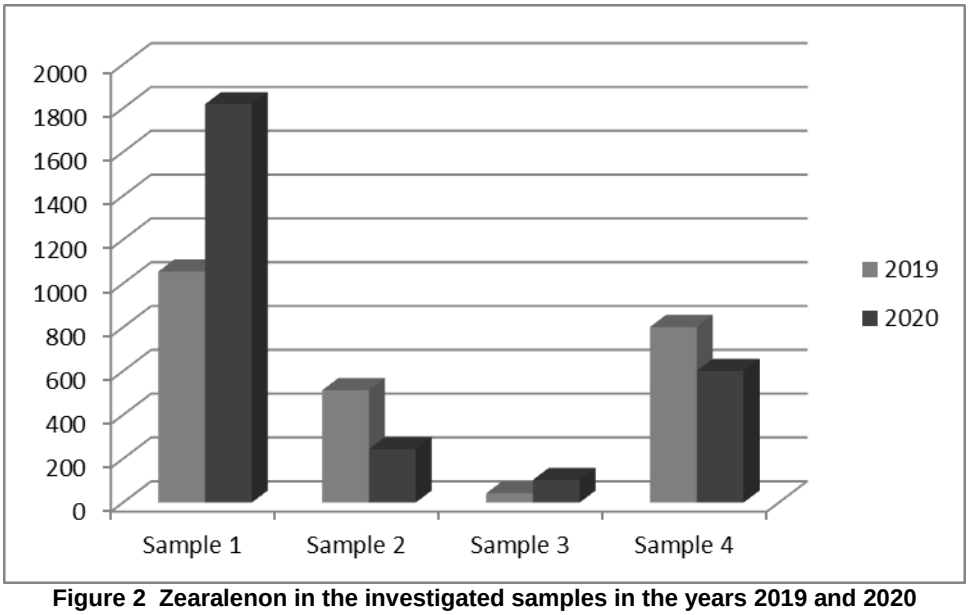

Figure 2 Zearalenon in the investigated samples in the years 2019 and 2020

Table 1

Contamination with Total aflatoxin and zearalenon in the investigated samples analysis

	Total aflatoxin – 2019	Total aflatoxin - 2020	Zearalenone - 2019	Zearalenone -2020
Mean value	1.13825	1.2215	602.4025	691.575
Median value	0.921	1.0935	656.875	422.31
Maximum	2.35	2.26	1054.03	1818.23
Minimum	0.361	0.439	41.83	103.45
Stdev	0.893	0.806	434.353	779.77

This negatively influences this branch of milk processing by decreasing milk production and the impact that mycotoxins have on animal health (Kemboi D. et al., 2020) Several cases have correlated zearalenone with the estrogenic response and severe fertility problems in cows,

even abortions. Symptoms include vaginitis, vaginal discharge, poor reproductive performance and enlargement of the mammary gland in virgin calves. (Smith G., 2019) The results of the analyzes of the gross chemical composition of the fodder samples from 2020, show higher values of the dry matter of the hay compared to 2019, with values of crude protein of almost 8.5%. There is an improved value of the crude protein value in corn silage compared to the dry matter of over 6%, but still below the average of 8%. Regarding the supplemented pelleted compound fodder, its nutritional value is higher, finding values of crude protein of almost 26.5%. We can appreciate that the nutritional value of fodder in 2020 is higher than in the fodder samples in 2019. For the determination of Zearalenone, samples were taken from each batch of fodder in 2020 and was found in all samples examined, 2 of the 4 exceeding the maximum permitted values in the feeding of dairy cows. Zearalenone values ranged from 103.45 µg / kg to 1818.23 µg / kg. The contamination is lower than the analysis from 2019 in 3 of the samples, the feed with the highest concentration of Zearalenone being the corn silage, exactly as in the 2019 sample, with a concentration of 1818.23 µg / kg, and the lowest concentration is in complementary feed, as in the 2019 analysis with 103.45 µg / kg. The measurements carried out in 2019 and 2020 show that the processing and storage of fodder is inadequate animal health and milk production being also affected. The farmer was advised to add mycotoxin inhibitors to feed. The addition of mycotoxin inhibitors in contaminated feed is considered the best method to reduce the effects of mycotoxins (Galvano et al., 2001). Activated carbon is considered a substance that binds very well to zearalenone and deoxynivalenol (Whitlow, 2014). The chemical structure of zearalenone is similar to that of estrogen. ZEA intoxications lead to reproductive problems, including symptoms of estrus in calves before puberty, irregular heat, silent heat, emission resorption, abortions, placental retention, metritis and mastitis (Obremski et al., 2012). European Community directives on the presence of deoxynivelenol, zearalenone, ochratoxin A, T2, HT2 and fumonizine; the zearalenone content must not exceed 250 µg / kg in the case of pig feed and a maximum value for cows and calves of 500 µg / kg. For young animals the values are much lower, 5 µg / kg for calves and lambs (EC, 2006). The productivity of dairy cows can be severely impaired by the presence of zearalenone in feed. Diagnosing and isolating zearalenone can sometimes be difficult and even stressful. The diagnosis of zearalenone poisoning and the induction of estrogenic effects are based on clinical signs and the detection of zearalenone in feed. Treatment is based on removing contaminated feed from animal feed and replacing it with high-quality feed. Although the toxicity of zearalenone varies quite a bit, farmers and veterinarians should take into account the estrogenic effects of this substance and the repercussions on the reproductive health of cows (Witte, 2003). In determining the aflatoxin, each feed sample from 2019 was analyzed separately. Aflatoxin was detected in all samples examined, but none exceeded the maximum values allowed in the feeding of dairy cows by European legislation. The determined values were between 0.361 µg / kg and 2,35 µg / kg in 2019 and between 0.439 µg / kg and 2.26 µg / kg in 2020. Contamination with total aflatoxin was in the investigated years constant, the average varying in a small margin. The maximum values of total Aflatoxins in the samples analyzed by us reveal values much lower than in other countries, such as Pakistan where values of up to 15 µg / kg for wheat and 13 µg / kg for maize were recorded (Lutfullah, 2012). Aflatoxins are very toxic to animals and humans. Even in non-lethal amounts, aflatoxins can endanger animal health and productivity. For dairy cows it should not exceed 20 µg per ration (Jordan, 2012). In a study to test the theories by which mycotoxins can bind to other compounds, activated carbon was added in high doses and shown to reduce aflatoxicosis in goats (Hatch et al., 1982).

CONCLUSIONS

The organoleptically analyzed feeds ranged from good to very good quality. The raw chemical composition of the fodder showed values quite close to those cited in the literature, except for the low values of crude cellulose in hay and a low value of crude protein in corn silage. Correlating the values of the gross chemical composition for the two years, we can appreciate that the nutritional value of the feeds in 2020 is higher than in 2019. Total aflatoxin and zearalenone were identified in all analyzed samples. Only zearalenone exceeded the maximum admitted limit in European Union in the majority of samples.

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SURVEY OF DOG AND CAT ANESTHESIA IN UNIVERSITY VETERINAY HOSPITAL PROFESSOR ALIN BÎRȚOIU

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Abstract

The aim of this study is the survey of current anesthesia cases and outcomes for dogs and cats in the University Veterinary Hospital Professor Alin Bîrțoiu, Bucharest, during 12 months, in order to improve anesthesia safety and education. Electronic medical records for patients were examined and 944 cases of dogs and cats that underwent general anesthesia (including sedation) assigned for ASA I-V risk classes, including emergencies were selected. The total mortality rates considered anesthetic-related death in dogs and cats (deaths in the first 48 hours attributable to anesthesia) were respectively 0.29% for dogs and 0.76 % for cats. The species, rase, sex and age structure of the case log were analyzed according to the type of the technique (injectable 12% or inhalation 88%). All the cases were preanesthetic evaluated and monitored during anesthesia and recovery.

Key words: anesthesia, survey, veterinary

The aim of this study is the survey of current anesthesia practices and outcomes for dogs and cats in the University Veterinary Hospital Professor Alin Bîrțoiu, Bucharest and to compare the results with other studies cited in literature. Large veterinary multicenter studies defined anesthesia-related death, for small animals, as occurring within 48 h after a procedure, where anesthesia could not be excluded as being one of the contributory factors, with an overall 4% in cats (Portier, 2020). Other studies are presenting lower anesthetic-related death (ARD) rate as 0.11% for cats and 0.05% for dogs (Matthews, 2017).

A multi-center small animal practice-based study, was undertaken in the UK and 98,036 anesthetics and sedations were recorded in dogs and 79,178 in cats (Brodblet, 2008). The risk of ARD was approximately 0.17% in dogs and 0.24% in cats, respectively 0.05% and 0.11% in healthy dogs and cats (ASA 1–2) versus >1% in sick patients (ASA 3–5).

Increasing age was associated with increased odds of death for both species. The results may be useful for the improvement of the anesthesia techniques in order to reduce anesthetic-related death.

Having the profile of the most frequent patients and results regarding their vital prognosis after anesthesia, can help us to adjust the protocols in order to decrease the risks and the mortality and to continue to study all the factors involved in peri-anesthetic period.

MATERIAL AND METHOD

This study was conducted on 944 cases of dogs and cats that underwent general anesthesia (including sedation) assigned for ASA I-V risk classes and emergencies. The cases were presented in the University Veterinary Hospital Professor Alin Bîrțoiu, Bucharest, during 12 months.

The ratio between canine and feline cases was 72.03%/ 27.97%, ages between 9 months and 17 years. Pre-anesthetic evaluation was performed for all cases in order to identify individual risk factors and to classify the risks prior to anesthesia.

Patient anesthesia risk was assessed according to American Society of Anesthesiologists (ASA) physical status classification system modified from the American Society of Anesthesiologists (Costea, 2016), shown to be a valuable prognostic tool, recommended to identify an increased risk of ARD until 24–72 hours after anesthesia (Portier, 2018). Checklists were used for each case., as they are shown to decrease ARD and reduce complications in veterinary practices (Haynes, 2009; Bergstrom, 2016). Various injectable (total intravenous anesthesia, intramuscularly sedation) and gaseous techniques, maintenance with Isoflurane) were used for the cases admitted in this study, according to the individual characteristics and procedures.

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Injectable protocols were used exclusively for unique sedation in ASA I-II cases or for general anesthesia in ASA I cases (normal healthy patient, with no organic disease- neutering).

Inhalant anesthesia protocols were used for ASA II-V (patient with mild systemic disease, severe systemic disease compensated/ decompensated, moribund patient) and emergency cases that require general anesthesia.

RESULTS AND DISCUSSIONS

This study covers the case portfolio of anesthesia (canine, feline) during 12 months, at the University Veterinary Hospital Professor Alin Bîrțoiu, Bucharest, which can be described as a referral emergency center for the entire south area of Romania. Most of the cases from this study are referred from small clinics or may come directly as emergencies or as critical cases. Another part of cases is referred for advanced imaging diagnostic (magnetic resonance imaging, computed tomography, radiology, ultrasound) that requires in some situation sedation or general anesthesia.

The age structure (Table 1) for the 944 cases of dogs and cats is presented in Table 1. For dogs the largest number of cases were included in the age interval 6-12 years (41.67%) while for cats the largest interval was 1-6 years (46.43%). The number of cases over 12 years of age in cats exceeded the dog's category (14.28% over 9.72%).

Regarding breed distribution of cases (Table 2), 12.2% of dogs were registered as Metis (canine mixed breed), while for cats 64.29 % were European (feline mixed breed). A group of 13.95% of dog were brachycephalic breeds (French Bulldog, Pug), known to have a high anesthetic risk. Brachycephalic cats, also considered difficult patients for anesthesia, represented 14. 28% of the cases (British shorthair, Scottish Fold). All the brachycephalic cases were intubated for general anesthesia (cases ASA II-IV).

Comparing the total number of cases for this survey, the results showed that 88.03 % of the techniques represented gaseous (inhalation) anesthesia, compared with 11.97 % injectable protocols. 8.24 % (56) of dog's cases and 21.59 % (57) of cat's cases represented injectable protocols (Table 3).

The higher percentage of injectable protocols in cats compared with dogs can be corelated with the higher number of sedations required, due to their aggressivity or lack of compliance during clinical procedures.

From the total number of cases, injectable anesthesia protocols represented 11.97 % (113). No ARD cases were recorded for this category of cases: young ASA I patients, anesthetized for elective sterilizations or sedations for ASA I-II.

ASA cases higher than III can have a significantly increased risk of complications, since the ASA status rather than age is a better predictor of peri anesthetic complications (Hosgood, 2002).

A total of 88.03% (831) represented inhalations protocols for ASA II-V patients (patients with different types of pathologies) and emergency cases, respectively 91.76% (624) of dog's cases and 78.41 % (207) of cat's cases (Table 3).

The ARD cases represented a total of 0.42% from the entire number of cases and respectively 0.29% for dog's cases and 0.76% for cat's cases (Figure 1).

All of the ARD cases were anesthetized by inhalation protocols and represented ASA II-V and emergency cases. Breed was not one of the factors involved in the ARD for these 4 cases (Table 1).

Differentiated results by species are not favorable for cats, were ARD recorded were higher than for dogs (0.97% versus 0.32%) and similar to other studies. Differences between ARD percentages for dogs and cats are corelated with the possible complications during anesthesia that are more common in cats than dogs. Data from bibliography (Brodblet, 2008) present results of >1% ARD for patients with different pathologies compared with this study's results, that show lower ARD for the group of cases ASA II-V and emergency cases (0.48%).

The results of this study show an increased rate of ARD for the total number of cases and types of anesthesia, compared with literature data, but important differences are observed between the specific of cases according to different types of cases covered (primary care veterinary practices, basic clinics, referral centers, teaching hospitals, referrals, emergency hospitals).

Table 1

Cases- age structure

Canine cases age interval/ %		ARD canine	Feline cases age interval/ %		ARD feline
0-12 months	12.5%		0-12 months	2 %	
1-6 years	36.11%	1 case-neurological case MRI scan (French Bulldog, ASA III)	1-6 years	46.43 %	2 cases- acute traumas (European cats, ASA IV)
6-12 years	41.67%		6-12 years	14.29 %	
12-15 years	9.72 %	1 case- pyometra (Pekinese, ASA IV)	12-15 years	10.71%	
			15-18 years	3.57%	
From 680 cases		**0.29%**	From 264 cases		**0.76%**
TOTAL 944 cases	**0.42%** (4 cases)				

Table 2

Cases- breed structure

Canine		Feline	
Metis	12.20%	European	64.29%
Havanese Bichon	11.11%	British Shorthair	10.71%
French Bulldog	9.72%	Sphynx	7.14%
Maltese Bichon	5.56%	Birman	5.14%
Beagle	4.27%	Maine Coon	3.14%
Pug	4.23%	Scottish Fold	3.57%
Labrador Retriever	4.21%	Other breeds < 2%	
Other breeds < 2%			

Table 3

Injectable versus inhalation cases and the mortality cases (ARD)

Cases	Injectable		Inhalation	
Canine (680)	8.24 % (56)	0 ARD	91.76% (624)	0.32% (2 ARD from 624 cases)
Feline (264)	21.59 % (57)	0 ARD	78.41 % (207)	0.97% (22 ARD from 207 cases)
TOTAL (944)	11.97 % (113)	0 ARD	88.03% (831)	0.48% (42 ARD from 831 cases)

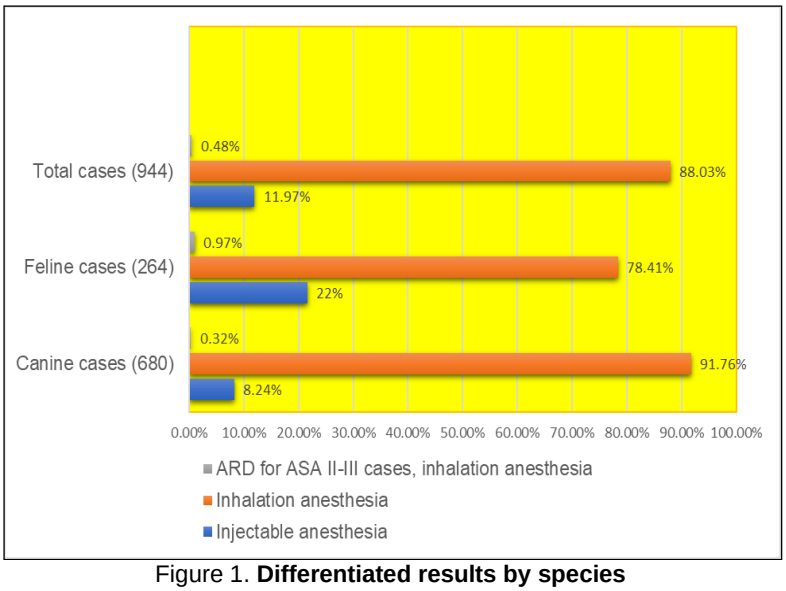

Figure 1. **Differentiated results by species**

CONCLUSIONS

The results obtained in this study describe the type of anesthesia techniques used for different species (canine, feline) breeds and age groups, as well as the mortality rate associated with anesthesia, in an emergency veterinary service.

Given that most of the dogs in the group are in the age range 6-12 years (41.67%) and brachycephalic breeds accounted for 13.95% of canine cases, the ARD rate was 0.32% and recorded exclusively in patients with acute pathologies (ASA III, ASA IV). None of the ARD were related to any breed risk factor for anesthesia. Brachycephalic cats, also considered difficult patients for anesthesia, represented 14. 28% of the cases and no ARD were recorded for this category.

From the total number of cases, injectable anesthesia protocols represented 11.97 % (113 cases ASA I, ASA II) compared with 88.03% (831 cases ASA II-V, emergency) inhalations protocols. For feline patients it was recorded a higher number of injectable protocols compared with canine patients (21.59 % versus 8.24 %), correlated with the higher number of sedations required for diagnostic procedures in their case while canine cases were subjected to a higher percentage of general anesthesia compared to injectable sedations.

All of the ARD cases were anesthetized by inhalation protocols for ASA II-V and emergency cases and represented a total of 0.42% from the entire number of cases (0.29% canine, 0.76% feline), respectively 0.48% only for the inhalational group, with a higher percentage for feline patients (0.97% versus 0.32%).

Anesthesia for patients with different pathologies in University Veterinary Hospital Professor Alin Bîrțoiu, Bucharest, registered lower ARD compared with other studies (0.48% versus 1%). The ARD for the total number of cases and types of anesthesia, should also be analyzed considering the important differences between the specific of cases of an emergency referral hospital compared with other types of studies and veterinary practices.

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STUDY REGARDING THE CHANGES IN SOME HEPATIC PARAMETERS DURING GENERAL ANESTHESIA IN A GROUP OF DOGS

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Abstract

The main organ involved in the biotransformation of the anesthetic drugs used for general anesthesia is the liver. Drugs will be at this level transformed into more easily excreted substances known as metabolites.The evaluation of the hepatic function before general anesthesia is essential for a safe and individualized protocol.Comprehensive understanding of the anesthetic drugs and their effects on hepatic functions during anesthesia remains fundamental.Techniques and protocols used for anesthesia and intensive care in the recovery phase will be designed taking into account the trend of the hepatic parameters.This study will focus on how the general anesthesia influences hepatic parameters measured before premedication and in the early recovery phase: alanine aminotransferase (ALT), alkaline phosphatase (ALP),total proteins (TP) and albumin (ALB). Following the result of the study,we discovered that ALP increased with 11% and ALT decresed with 12% after general anesthesia in comparison with the value before premedication.

Key words:general anesthesia,liver,biotransformation

INTRODUCTION

This study will present the changes that appear on some hepatic parameters during general anesthesia in a group of dogs.The liver is the central organ involved in the metabolism of the anesthetic drugs.The blood that arrives from the gastrointestinal tract is full of proteins,carbohydrates,fats and other exogenous particles(drugs,bacteria).From the total cardiac output,25-30%flows through the liver via dual blood supply: the hepatic artery and the portal vein (Grimm, K. A. *et al*, 2015).

Liver has multiple functions,among which the biotransformation of anesthetic drugs is the most important.The hepatic parameters that will be taken into consideration in this study are: alanine aminotransferase (ALT), alkaline phosphatase (ALP),total proteins (TP) and albumin (ALB).

TP is a biochemical test for measuring the amount of proteins in the blood plasma.They are mainly produced by the liver and they have multiple functions,including transport of lipids, vitamins, hormones and minerals.Some blood proteins have different functions and can act as enzymes or protease inhibitors.Here we can list just a few types of proteins: albumins, globulins or fibrinogen.

ALB is the main total protein and accounts around 55%of it..It has a major contribution on maintaining the oncotic pressure of plasma and acts as a major carrier for insoluble molecules.The majority of anesthetic drugs has insoluble molecules,so the level of ALB needs to be within normal range.

ALT is a hepatic enzyme and is found mainly in the hepatocytes and in a small quantity in other organs(kidney,skeletal muscles).Elevation in ALT occure secondary to leakage from the hepatocytes after the damage of the hepatocyte membrane.(Ettinger S, *et al, 2017*).ALP is a membrane bound glycoprotein that hydrolyzes phosphate esters.In dogs and cats it is produced by the liver,renal cortices,intestine and placenta.(Nelson R, Couto C, 2019).

For the blood tests we used the veterinary biochemical analisator SMT-120V.The operating system is based on the spectrometry tehnique.The sample used for this machine can be serum,plasma or blood and it needs to be collected in lithium heparin tubs.

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Fig 1.Biochemical lab analisator SMT-120V

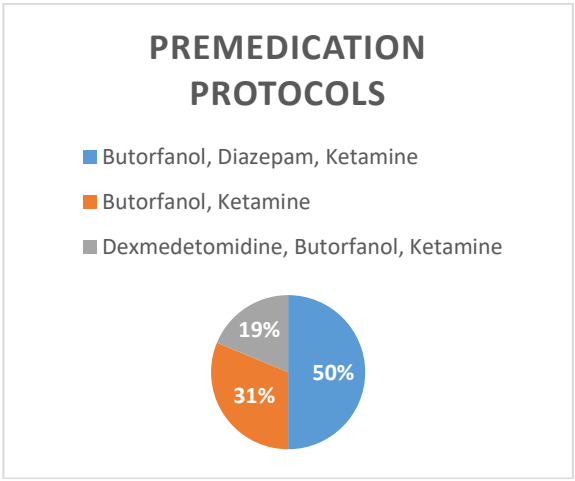

Fig 2.Premedication protocols used in the study group

MATERIALS AND METODS

This article represents a clinical study conducted on 16 canine patients,with age between 1 and 12 years old.The study was conducted by The Veterinary Medicine Faculty Bucharest and will describe how hepatic parameters change before and after general anesthesia.A preanesthetic exam was performed for each patient,including blood tests and full cardiac exam.Based on this results,the patient was assigned an ASA status(American Society of Anesthesiology).We selected the patients that were included in II-III ASA score. Patients underwent different types of surgeries,including ophtalmological surgeries (cataract and intrasclerotic prosthesis) and urogenitalsurgeries (ovariohysterectomy, cystotomy or perineal hernia). Depending on the procedure type ,the time the animal spent under anesthesia was different.For example,the opthalmological procedures were shorter and did not require a very deep anesthesia plan compared with the urogenital ones.

Depending on surgery types and the expected pain level,we used multiple anesthetic protocols..All of them included Propofol (2-5 mg/kg,IV) for induction, intubation and maintenance with Isoflurane in 100%oxygen.Ringer solution was administrated throughout all the surgery,on a rate of 3-5 ml/kg/h.

Based on the premedication,we can divide the study group into 3 categories:first group was premedicated with Butorfanol (0.2 mg/kg),Diazepam (0.2 mg/kg)and Ketamine (2 mg/kg), second group with Butorfanol (0.2 mg/kg) and Ketamine (2 mg/kg) and the thrid group with Dexmedetomidine (2mcg/kg), Butorfanol (0.3 mg/kg)and Ketamine(2 mg/kg), as presented in Fig 2.

The patients of this study were also divided into 2 groups based on their age:first group includes patients with age from 1 up to 6 years old and the second one patients from 7 to 12.

For the purpose of this study,the before and after biochemical tests were taken on the same lab device for better results comparison..The parameters that we took into account for this study are ALT,ALP,TP,ALB.

RESULTS AND DISSCUTIONS

The results suggested an increase with 11%in the ALP value,respectively 19%in ALB value and a decrease with 12%in ALT value,respectively 2%in TP value within the hole group of dogs.

For the group age 1 to 6 years old,we noticed an increase with 33.04%in ALP value,respectively 25.43%in ALB value and a decrease with 30.03%in ALT value,respectively 4.82%in TP values.

In dogs with age between 7 and 12 years old,we noticed only an increase values compared with the values obtained before premedication: 6.66%in ALP, 7.71%in ALT, 12.65 in ALB value and 0.38%in TP value.All this values can also be found in the table Fig.3.

As seen in the study,all the parameters in the age group 7 to 12 years increased.This can be explained by the age and the fact that the liver has reduced metabolic functions.On the other hand,in the 1 to 6 years old group,there was a significant increase in the ALP values,respectively a decrease in ALT values.

In human medicine, serum liver enzymes have a significantly increase during the first 48 hours after laparoscopic cholecystectomy and laparoscopic colorecal cancer resection (Tan, M.*et al*, 2003). Also, other studies showed that both total

intravenous anesthesia as well as inhalatory anesthesia are useful and can be used for patients that have elevated liver enzymes values.Both types of anesthesia may determine a transitory elevation in the liver enzymes,but this will not severely affect the liver function (Oh S. *et al*, 2020)

CONCLUSSIONS

In conclusion,there are differences in the hepatic parameters that were taken into account for this study(ALT,ALP,TP,ALB).The parameters were measured before premedication and in the early recovery phase.This modifications can be associated with the age of the patient,the type of the surgery and the duration of the anesthesia. Further studies will be continued in order to test bigger groups on different protocols.

Date from the hole study group				
Row Labels	**Average of ALP(U/L)**	**Average of ALT(U/L)**	**Average of ALB(g/dl)**	**Average of TP(g/dl)**
After	183.08125	72.75	2.98125	6.63125
Before	203.6875	64.36875	3.54375	6.48125
Difference	**11%**	**-12%**	**19%**	**-2%**

dogs with age between 1-6 years				
Row Labels	**Average of ALP(U/L)**	**Average of ALT(U/L)**	**Average of ALB(g/dl)**	**Average of TP(g/dl)**
After	63.75	74.125	2.9	6.7375
Before	84.8125	51.8625	3.6375	6.4125
Difference	**33.04%**	**-30.03%**	**25.43%**	**-4.82%**

dogs with age between 7 and 12 years				
Row Labels	**Average of ALP(U/L)**	**Average of ALT(U/L)**	**Average of ALB(g/dl)**	**Average of TP(g/dl)**
After	302.4125	71.375	3.0625	6.525
Before	322.5625	76.875	3.45	6.55
Difference	**6.66%**	**7.71%**	**12.65%**	**0.38%**

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EPIDEMIOLOGICAL RISK OF TOXOCAROSIS IN HUMAND AND ANIMALS IN IAȘI COUNTY

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Abstract

The increasing number of dogs is a determining factor in the occurrence of toxocarosis in humans, as they are the source of environmental contamination with *Toxocara sp*. eggs. During a single year, 2018-2019, the number of stray dogs increased from 0.068 to 0.0709 per capita. Contamination of dogs with *Toxocara canis* in the conditions of our country has increased in recent years from 21.4% to 50.2% and is identified as the most common parasitosis in these animals. For the study of *Tococara spp*. infection in dogs, the period 2017-2020 was considered, representing cases present at the Faculty of Veterinary Medicine, Iasi. Thus, more than 75% of infections are recorded in young dogs under one year old, while 89% of them are males. The study on the prevalence of *Toxocara canis* cases at the Animal shelters in Tomești showed a prevalence of Toxocara sp. of 60% of the total samples analysed; the study on toxocarosis in humans was carried out during 2020, the information being provided by the Praxis medical tests laboratory. Result on the presence of specific IgG antibodies to *Toxocara canis/cati*. It included a group of 95 cases during one year, of which 3, namely 3.25% were under 3 years old and 14.8% were over 35 years old. Of the total samples, only 2 were positive in the male gender, which represents 2.1% of the total samples. Fifteen cases were positive in females, representing 15.8% of all samples analysed. Considering that we are talking about a parasite specific to dogs, the presence of such a large number of cases during a single year reveals a very high load of *Toxocara* eggs in the environment, which raises an alarm about the distribution of this parasite in nature and the high risk of human contamination.

Keys words: toxocarosis in humans**,** environmental contamination

Toxocara canis, Werner (1782), is an ascarid that parasitizes domestic dogs (Canis familiaris), its morphology being similar to that of the nematode *Ascaris lumbricoides* (parasite of man), adult males are 4-10 cm long and females are 6.5-18 cm long.

The disease caused by this parasite is called toxocariasis, and mainly affects dogs, but can also affect other animals. In humans, the parasite can also cause this disease, which, if not treated in time, can trigger very serious consequences.

Toxocariasis is a zoonotic disease of great importance in terms of the morbidity it can cause in humans and animals, but also in terms of the danger it poses to their health. Recent findings on its association with other pathologies, advances in diagnostic techniques and new therapeutic discoveries raise the concern to review a current topic that may be considered forgotten and neglected due to the lack of national and European studies. *Toxocara canis* can affect humans, causing the so-called *larva migrans visceralis* and *larva migrans ocularis* syndromes. Human illness is due to egg ingestion, with children being more susceptible due to poor hygiene. Spaces shared by dogs and children, such as parks, can become sources of contamination; in this respect, it is essential that dog faeces are always collected.

The life cycle of the *Toxocara canis* parasite in humans is different from that in dogs, as the larvae cannot reach the adult stage. Furthermore, they pass through the intestinal wall and migrate to the liver, lungs and skin. In massive infestations, symptoms such as abdominal pain, coughing, itching or rashes occur and the larvae can spread to the heart, kidneys, spleen, brain or eyes.

Prevention in humans involves proper deworming of dogs and cats and educating children about basic hygiene. The main source of transmission is puppies shedding large amounts of eggs (Despommier, 2003; Manson et al., 2003). Infection is acquired mainly by children when playing on contaminated soil or in parks, similar to what happens in *A. lumbricoides* infection, and also occurs in association with the phenomenon of soil ingestion.

Direct infection by handling animals is not considered a major risk as *T. canis* eggs excreted into the environment require at least two weeks to become infested by the host (Manson et al., 2003;

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Overgaauw, 1997). In addition to dogs and cats, other animals, especially peridomestic animals such as squirrels, rabbits and other small and medium-sized mammals, can play an important role in the dispersal of embryonated eggs (Despommier, 2003; Dubinsky et al., 1995). Birds that feed primarily on the ground (such as pigeons or sparrows) may be paratenic hosts, but may also carry eggs from place to place on their feet or wings and may be responsible for laying eggs in distant locations (Hoffmeister et al., 2007; Morimatsu et al, 2006; Taira et al., 2003). Another mechanism of egg dispersal is the consumption of contaminated water (and food, especially vegetables), which has been demonstrated in some studies (Despommier, 2003; Doligalska & Donskow, 2003; Schwartzbrod & Banas, 2003; Vazquez Tsuji et al., 1997).

Larva migrans visceralis syndrome

In intense infections, especially in children (under 5 years of age), juvenile larvae, measuring on average 450 μm x 16-20 μm in diameter, occur mainly in the liver, where they may cause fewer or more miliary lesions, and foci of necrosis may even occur (Despommier, 2003; Manson et al., 2003).

The clinical picture accompanying this pathology includes fever and lower respiratory tract symptoms (especially bronchospasm, reminiscent of asthma) with eosinophilia (which may even reach figures close to 70% or greater than 10,000 cells/mm³) and hyperglobulinaemia (IgM, IgG and IgE) (Pinelli et al., 2007).

Macroscopically in the liver, lesions consisting of granulomas that can be described as white subcapsular nodules the size of millet seeds are observed, but an increase in liver volume can also be observed.

Depending on the organ parasitized, it can cause myocarditis, nephritis, central nervous system damage, convulsions, neuropsychiatric symptoms and encephalopathy (Despommier, 2003). Experimental neuropathogenicity studies conducted more than 30 years ago identified that *T. canis* larvae move actively in the brain, penetrating directly through tissues as well as moving back and forth in the brain through the meninges and ventricular space (Innes & Saunders, 1962), being observed in some cases due to granuloma formation and producing both the pathologies described and manifested clinically and neurologically by their passage as well as by the latter.

The clinical manifestations of occult toxocariasis are variable and may present as a picture of pulmonary disease (asthma, bronchitis, pneumonitis), dermatological disorders (chronic urticaria or eczema), lymphadenopathy, myositis and pseudo-rheumatic syndromes such as arthralgia 2001).

Ocular larval migrans syndrome or ocular toxoplasmosis

In the eye, juvenile larval migrans can damage the retina by forming large subretinal masses and inducing granulomatous reactions, which can lead to decreased vision (Despommier, 2003; Manson et al., 2003). The ocular syndrome usually occurs in children aged 5 to 10 years and usually determines unilateral vision impairment, sometimes accompanied by strabismus (Molk, 1983; Taylor, 2001). In 2004, a premature infant was reported from a hospital neonatal intensive care unit, referred for treatment of retinopathy of prematurity, in whom a larval image in the retina of the left eye was found. These findings could be supported by previous studies postulating potential congenital transmission of intestinal nematodes (da Costa-Macedo and Rey, 1990).

MATERIAL AND METHOD

The study aimed to determine the prevalence of toxocariasis in humans and animals in Iasi County.

This study was prompted by the detection in the latest period in humans by practitioners, clinicians, specialists in various medical branches of an increasing number of patients with various clinical manifestations, in whom the presence of antibodies specific to the parasite *Toxocara canis* was detected by serological tests and a series of specific investigations.

At the same time, another category of people with subclinical or even absent signs of the disease has appeared, who, after a series of tests, approach the doctor with positive anti-*Toxocara* serological tests. As a rule, the vast majority of these people did not have a medical response to the interpretation of the positive results and consulted several specialists several times.

There is a gap among clinicians with regard to the recognition of clinical manifestations but also with regard to the management of toxocariasis, as well as among the population with regard to prevention and control measures for this disease.

The research was carried out in two locations: Ion Ionescu de la Brad University of Life Sciences in Iasi, MV Parasitology Clinic, Animal Shelters Tomești and Praxis Human Medical Tests Laboratory.

In the last period, molecular biology and bioinformatics have developed more and more, but one thing has remained constant, namely the examination for the diagnosis of endoparasites,

which is based on the coproparasitological examination, namely the analysis of faecal samples to determine the presence of parasites or parasitic elements; this is the best known procedure to correctly and concretely diagnose an endoparasitosis.

For the diagnosis of toxocariasis in animals, the *Willis flotation method* has been used which is based on the principles of differentiating between the specific weight of parasite eggs and faecal remaining. Salt or sugar at 30% concentration is used as flotation solution.

During 2020 at the Faculty of Veterinary Medicine 180 faecal samples were analysed, of which only 45 samples were positive for the presence of the parasite *Toxocara canis*. Of the positive cases 75% were identified in dogs aged between 2 months and 1 year, the remaining cases were identified in dogs over 14 years of age.

RESULTS AND DISCUSSIONS

Results of study at the Faculty of Veterinary Medicine

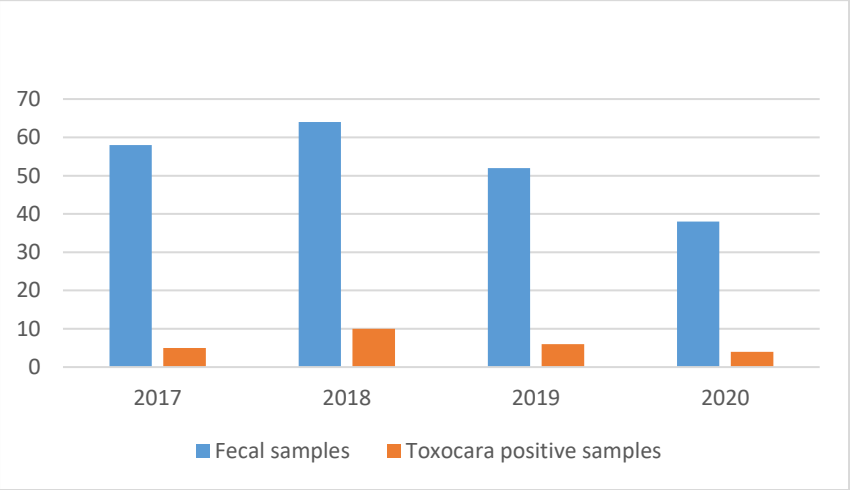

Figure 1 Positive results by Toxocara sp.

During the years 2017-2020 at the Faculty of Veterinary Medicine, 212 faecal samples were analysed, of which only 25 samples were positive for the presence of the parasite *Toxocara canis (figure 1)*.

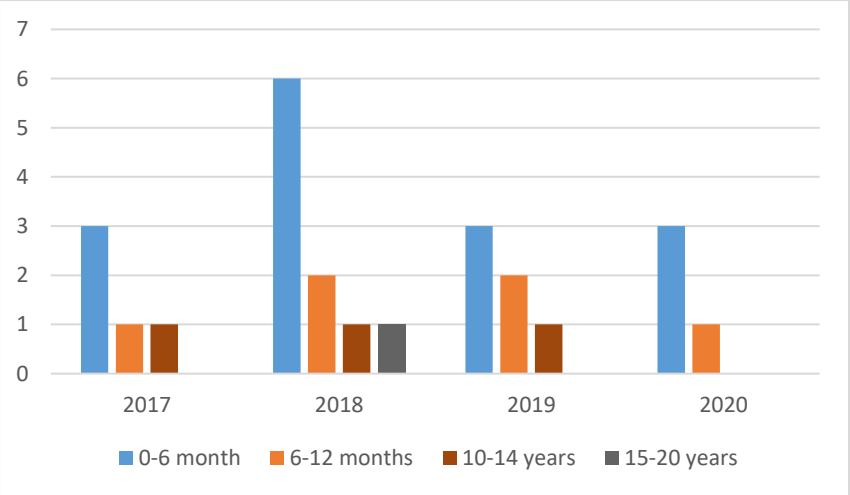

Figure 2 Distribution of Toxocara sp. by age groups

Of the positive cases 84% were identified in dogs aged between 2 months and 1 year, the remaining

cases were identified in dogs over 14 years of age (*figure* 2).
Results of study at the Animal Shelters Tomesti

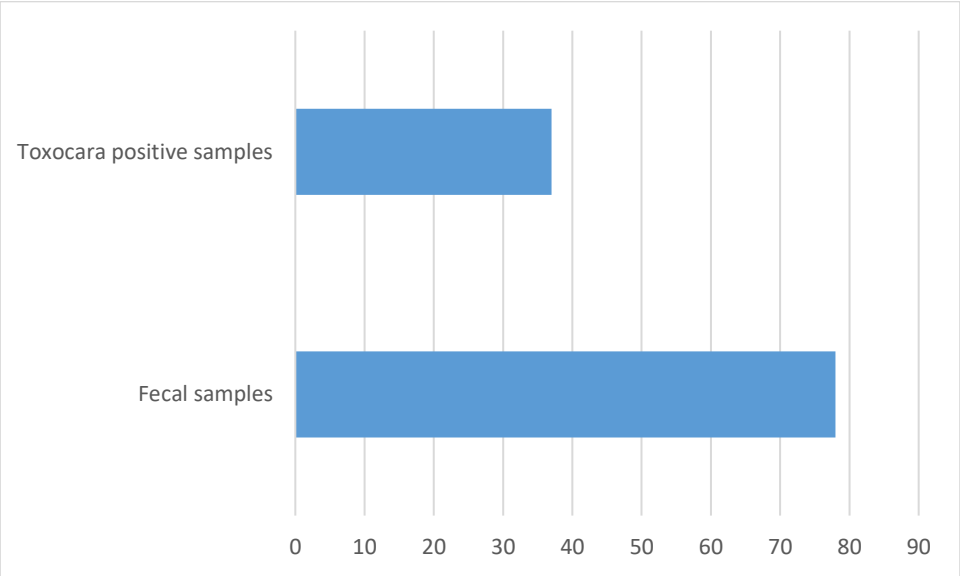

Figure 3 Positive results by toxocarosis at the Animal sheltes Tomesti

During 2020 from the Animal Shelters Tomesti, 78 faecal samples were analysed, of which 37 samples were positive for the presence of the parasite *Toxocara canis*. The faecal samples were collected without knowing the age of the animals (*figure* 3).

Table 1. Ig A antibody testing in the Praxis laboratory
Service Name/Category/Age Total Total negatives Total positives Positives Sex M Positives Sex F Total Sex F

Serviciu	DenumireCategVarsta	Total	Total negative	Total pozitive	Pozitive Sex M	Pozitive Sex F	Total Sex M	Total Sex F
Toxocara canis/cati - Anticorpi IgA	0-12 luni	0	0	0	0	0	0	0
	1 an	0	0	0	0	0	0	0
	2 ani	1	1	0	0	0	0	1
	3 ani	3	3	0	0	0	0	3
	4 ani	1	1	0	0	0	1	0
	5-9 ani	5	5	0	0	0	2	3
	10-14 ani	4	4	0	0	0	1	3
	15-19 ani	2	2	0	0	0	1	1
	20-24 ani	0	0	0	0	0	0	0
	25-34 ani	1	1	0	0	0	0	1
	35-44 ani	0	0	0	0	0	0	0
	45-54 ani	7	6	1	0	1	1	6
	55-64 ani	4	4	0	0	0	2	2
	65-74 ani	3	3	0	0	0	0	3
	75-84 ani	2	1	1	0	1	0	2
	85+ ani	0	0	0	0	0	0	0
		33	31	2	0	2	8	25

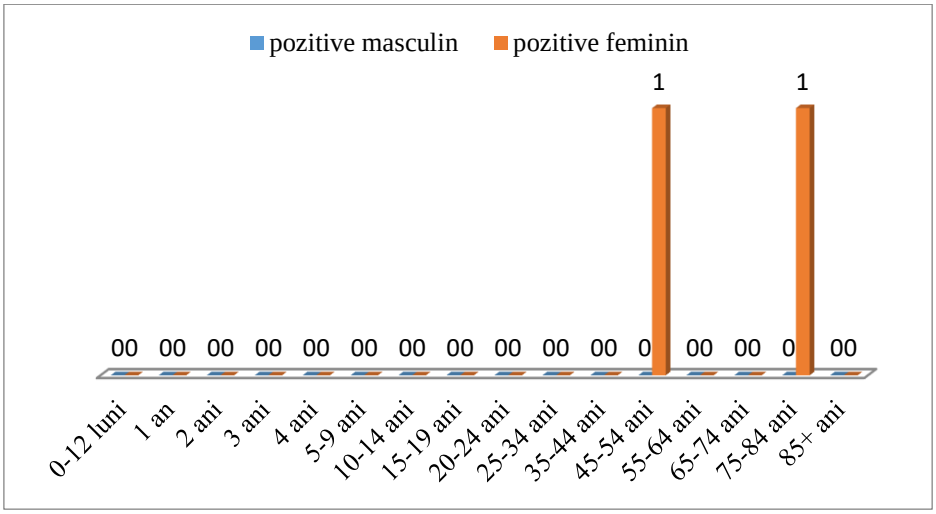

Figure 4 IgA antibodies -Toxocara canis(Positive males and Positive females)

Determination of Toxocara canis infection by IgA antibody study in the Praxis laboratory

Out of a total of 33 cases analysed, only 2 cases were positive, both in females, aged 45-54 years and 75-84 years respectively, representing 6.06% of the total cases studied. *Larva migrans* syndrome in humans is correlated with immunosuppression, the body being unable to stop the migration of larvae through the body, and thus the appearance of symptoms specific to the affected organ. Thus, in adults this disease is associated with chronic, immunosuppressive diseases such as AIDS or various forms of cancer, which means that the detection of the presence of *Toxocara* larvae must be accompanied by other tests to discover the main cause of the disease (*figure* 4)

Table 2. IgG antibody detection samples at the Praxis unit

Service Name/Category/Age Total Total Positives Sex Positives Sex Total Sex F

IgG antibodies

Serviciu	DenumireCategVar	Total	Total	Total	Pozitive Sex	Pozitive Sex	Total Sex	Total Sex F
Toxocara canis/cati - Anticorpi IgG	0-12 luni	3	3	0	0	0	3	0
	1 an	1	1	0	0	0	1	0
	2 ani	2	1	1	0	1	1	1
	3 ani	4	2	2	0	2	0	4
	4 ani	1	1	0	0	0	1	0
	5-9 ani	8	8	0	0	0	5	3
	10-14 ani	13	13	0	0	0	6	7
	15-19 ani	4	4	0	0	0	0	4
	20-24 ani	4	4	0	0	0	1	3
	25-34 ani	6	6	0	0	0	1	5
	35-44 ani	10	9	1	0	1	2	8
	45-54 ani	18	14	4	2	2	5	13
	55-64 ani	11	9	2	0	2	5	6
	65-74 ani	6	2	4	0	4	1	5
	75-84 ani	4	1	3	0	3	0	4
	85+ ani	0	0	0	0	0	0	0
		95	78	17	2	15	32	63

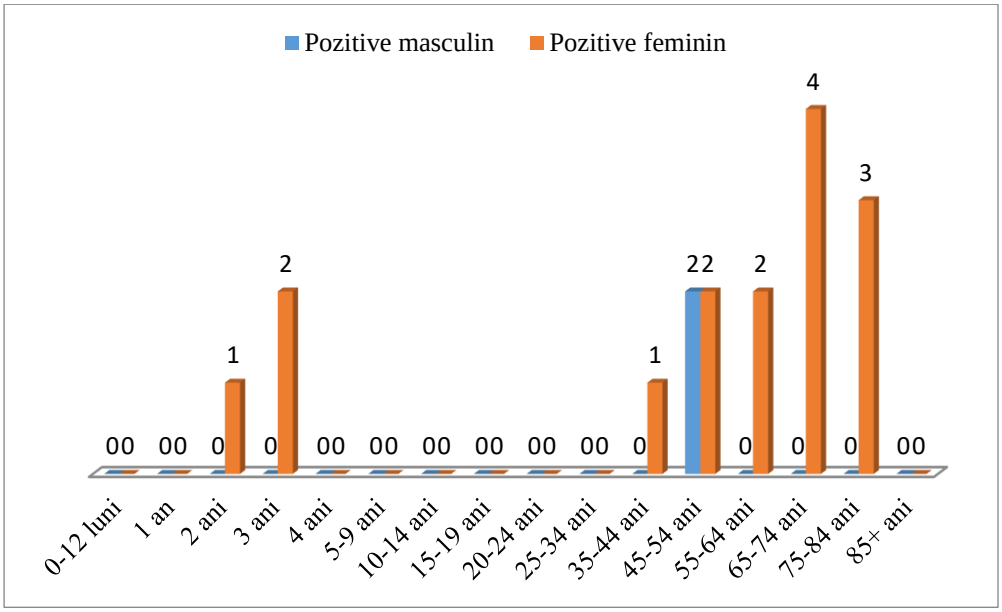

Figure 5 IgG antibodies-Toxocara canis(Positive males and Positive females)

Determination of Toxocara canis/cati infection by IgG antibody study in the Praxis laboratory

The above graph shows cases of *Toxocara canis* infestation detected by the presence of IgG antibodies to *Toxocara canis*. During a single year a total of 95 cases have been analysed, of which 3, namely 3.25% are under 3 years of age and 14.8% are over 35 years of age. Of the total samples, only 2 were positive in males, which represents 2.1% of the total samples. Fifteen cases were positive in females, representing 15.8% of all samples analysed. Considering that we are talking about a parasite specific to dogs, the presence of such a large number of cases during a single year reveals a very high load of *Toxocara* eggs in the environment, which raises an alarm about the distribution of this parasite in nature and of the high risk of human contamination (fig.5).

CONCLUSIONS

1. The increasing number of puppies is a determining factor in the occurrence of Toxoocariasis in humans, as they are the source of environmental contamination with *Toxocara* eggs. In a single year, namely 2018-2019, the number of stray dogs increased from 0.068 to 0.0709 per capita.
2. The number of dogs per 100,000 inhabitants in Iasi is 1.62 times higher than in Bucharest and 3.5 times higher than in Chisinau.
3. The contamination of dogs with *Toxocara canis* in our country has increased in recent years from 21.4% to 50.2% and is identified as the most frequent parasitosis in these animals.
4. For the study of infection with *Tococara spp*. in dogs, the year 2020 was considered, representing cases present at the Faculty of Veterinary Medicine in Iasi. Thus, more than 75% of the infections occur in young dogs under one year of age and 89% of them are males.
5. The study on the presence of specific IgG antibodies to *Toxocara canis* in humans was carried out during 2020, information provided by Praxis tests laboratory. It included a batch of 95 cases during one year, of which 3, namely 3.25% are under 3 years of age and 14.8% are over 35 years of age. Of the total samples, only 2 were positive in the male gender, which represents 2.1% of the total samples. Fifteen cases were positive in females, representing 15.8% of all samples analysed.
6. The study provides important information on the risk of *Toxocara canis* contamination in humans, contributing to public health, and the results obtained require prophylactic measures by controlling environmental infestation with *Toxocara* eggs.
7. The main source of contamination of children with *Toxocara* is the ingestion of embryonated eggs from the environment (parks, playgrounds); strict control of the access of ownerless dogs to such places for children is required.

8. In view of the presence of cases in adults, the risk of contamination with *Toxocara* eggs following the consumption of fruit and vegetables that have not been properly washed is highlighted, as the parasite's resistance in nature is high, of around 2 years.

9. The present study stresses the need to control stray dogs and to regularly deworm those with owners, as well as to inform and educate the population on hygiene measures and on the ways of parasite contamination.

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SEROLOGICAL DETECTION OF ANTIBODIES TO *EHRLICHIA CANIS* AND *BORRELIA BURGDORFERI S.L.* IN URBAN HOUSEHOLD DOGS FROM IAȘI

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Abstract
Canine tick-borne diseases are an emerging problem within Romania and also throughout the European Countries. This threat comes not just from Lyme disease which is endemic in our country, but also from other tick-borne diseases as well as ehrlichiosis. The present study consisted in screening of IgG class antibodies to *Borrelia burgdorferi* and *Ehrlichia canis* on 92 urban household dogs (48 females and 44 males) from Iași. The sampling took place during March to June 2021, in three veterinary clinics. The results of the serological testing revealed four positive dogs: one for IgG anti-*E. canis* and three for IgG anti-*Borrelia burgdoferi s.l.* Although the proportion of the sampled dog sex was almost equal, all positive animals were adult females. Our results highlight the silent circulation of the two pathogens in the studied area. These tick-borne pathogens are a significant medical concern to canine health. Changing tick distributions, pet travel and nonspecific clinical signs can make identifying infected pets challenging, so is very important to keep all dogs on appropriate, effective tick prevention year-round.

Key words: *Lyme borreliosis*, *Canine ehrlichiosis*, immunoassay, IgG antibodies

INTRODUCTION

Tick-borne diseases are a global emerging problem for both animals and humans. *Ehrlichia* and *Borrelia* are bacteria genus with some species bearing a zoonotic character (Springer et al, 2020). Lyme borreliosis is probably the most prevalent tick-borne disease in dogs. Its causative agents, Lyme spirochaetes from the *Borrelia burgdorferi sensu lato* complex are transmitted by *Ixodes ricinus* ticks in Europe (Leschnik, 2014). The most common ticks found on Romanian dogs continue to be *Ixodes spp* ticks (Mihalca et al, 2012; Păduraru et al, 2012), *Dermacentor reticulatus* being also present across the Eastern Romania (Brătuleanu et al, 2021). The *Ehrlichia* genus is comprised by six species, *E. canis* being one of them. All species of *Ehrlichia* infect vertebrate hosts, and all of them are transmitted by ticks. Canine monocytic ehrlichiosis is a hemoparasitosis caused by the intracellular bacterium *Ehrlichia canis*, which has a worldwide distribution, although it occurs more frequently in regions with temperate climates due to the high prevalence of its biological vector, the *Rhipicephalus sanguineus* (Carrade et al, 2009).

Animals suffering from TBDs may develop life-threatening conditions if early clinical signs are not recognized and treatment has not been promptly instituted. Most dogs with infections like borreliosis and ehrlichiosis are asymptomatic, and only about 5% to 10% of infected dogs develop clinical signs (Elsheikha, 2016). During the recent years tick borne diseases have been described more frequently. This is believed to be because of increased pet travel, better animal care from pet owners and increased interest of clinical practitioners to investigate unusual clinical signs.

MATERIAL AND METHODS

A total of **92** samples were collected during March to June 2021 from urban household dogs in Iași city (table 1). The serum samples originate from dogs examined for different pathologies (surgical or internal medicine) in three clinics (two private clinics and in emergency unit of FVM). After collection, the serum samples were stored at -20^{0}C until testing.

All animals were tested for detection of IgG antibodies to *Ehrlichia canis* and *Borrelia burgdorferi s.l* using two available ELISA kits from Euroimmun (Germany). The first immunoassay kit provides a semiquantitative in vitro detection for antibodies of the immunoglobulin class IgG against *Ehrlichia canis*, with a sensitivity of 92% and specificity of 100%. The second ELISA test provides a semiquantitative *in vitro* assay for canine antibodies of the IgG class against *Borrelia* antigens in serum or plasma. The test kit contains wells coated with a mix of whole antigen extracts of *Borrelia burgdorferi sensu stricto, Borrelia afzelii* and *Borrelia garinii*. The testing protocols followed the procedure recommender by the producer. The results were evaluated semiquantitatively by calculation the

ratio of extinction of the sample over the extinction of the calibrator.

Table 1.

Distribution of the tested animals by collection site, sex and age

Consultation place	No. of samples	Sex	Age category		
			0-5 years old	5-10 years old	>10 years old
Private veterinary clinic 1	28	14 females	6	4	4
		14 males	2	5	7
Private veterinary clinic 2	8	5 females	5	-	-
		3 males	3	-	-
Emergency unit of FVM	56	29 females	16	8	4
		27 males	8	14	4

RESULTS AND DISCUSSIONS

Rickettsial organisms are small, obligate intracellular bacteria in the order *Rickettsiales*. These pathogens are transmitted by a variety of tick vectors, maintained in wildlife and domestic reservoirs, and can cause clinical disease in humans, dogs, and other domestic animals.

Our study that consisted in serological testing of 92 household dogs for detection of IgG anti-*E. canis* Ab revealed one positive sample, a 13 years old mixed breed female and a sample with borderline result collected from a 7 years old male. A borderline result does not exclude an infection with *E. canis.* Particularly in the early phase of infection, antibodies may not yet be present or are only present in such small quantities that they are not detectable. Secondly, the results of the serological testing for IgG anti-*Borrelia* antibodies revealed three positive animals: a 13 years Bichon female, a13 years old and a 4 years old mixed breed female. All four positive animals for rickettsial tick-borne infections were adult females. Similar results were published in 2018 in a study conducted by Galay et al, were more females (37.5%) were found infected with at least one TBP than males (25%). Moreover, adult dogs are more likely to have had attached ticks that went unnoticed in the past, increasing their chances of exposure to tick-borne pathogens.

Our results highlight the silent circulation of the two pathogens in the studied area. All Rickettsia species known to infect dogs are zoonotic; hence our findings should raise an alarm because of the risk to humans (Chrome, 2011). Dogs have often been reported to serve as effective sentinel animals to assess the risk of human rickettsial tick-borne infection. These tick-borne pathogens are also a significant medical concern to canine health. Changing tick distributions, pet travel and nonspecific clinical signs can make identifying infected pets challenging, so is very important to keep all dogs on appropriate, effective tick prevention year-round.

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TOXOPLASMOSIS-A DISEASE WITH HIGH EPIDEMIOLOGICAL RISK IN HUMANS AND ANIMALS

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Abstract

Infections produced by the protozoan *Toxoplasma gondii* are widespread in humans and animals. Due to its lack of host specificity, this parasite is able to infect a large number of hosts as well as different cell types. Although toxoplasmosis is the most reported parasitic zoonosis in Europe, the incidence of the disease in humans and the presence of the parasite in animals, food and water is underestimated. If acquired as an acute infection during pregnancy, *Toxoplasma gondii* infection can have serious adverse effects on mothers, foetuses and newborns. Latent toxoplasmosis also causes a variety of pathologies and has been linked to serious adverse effects on pregnancy. The study was conducted over a 2-year period, 2019-2020, in the Parasitology Clinic of the Faculty of Veterinary Medicine, Iasi, following the prevalence of reported cases of toxoplasmosis in cats. Thus, out of 33 tests worked, no case of toxoplasmosis was recorded in cats, all serological tests being done upon request. During 2020, 226 AB. ANTI *TOXOPLASMA GONDII*- IgM (ELISA) tests were performed in the Praxis laboratory, of which only 15 were positive. All positive tests were identified only in women, of which 10 in the age category 25-34 years, 4 in the age category 35-44 years and 1 case in the age category 15-19 years. In the Praxis laboratory during 2020, 220 more AB. ANTI TOXOPLASMA GONDII- IgG (ELISA) tests were performed, out of which 72 positive cases were identified, 5 being positive in males in the age categories 0-12 months, 1 year and 15-19 years, and the remaining 67 were identified in women in the following age categories: 0-12 months, 15-19 years, 20-24 years, 25-34 years, 35-44 years, 45-54 years and 55-64 years. The lack of positive cases in animals during the 2-year study, but the high number of positive cases in humans during a single year, shows the major public health importance of the study, as this very serious disease in pregnant women and immunosuppressed people is under-diagnosed in veterinary medicine.

Keys words: *Toxoplasma gondii*, parasitic zoonosis.

Despite the fact that toxoplasmosis is the most reported parasitic zoonosis in Europe, the incidence of the disease in humans and the presence of the parasite in animals, food and water is underestimated (EFSA, 2007). In order to understand the molecular epidemiology of toxoplasmosis, it is necessary to study strains from different geographical areas. The first studies on *Toxoplasma gondii* strains were obtained in humans and domestic animals, mostly from the USA and Europe, based on single-cell analysis. No atypical or recombinant strains could be identified in the studies. The study of multiple markers can accurately determine circulating genotypes, because strains belonging to specific genotypes, but also non-specific strains showing atypical alleles can be identified by means of multilocus analysis. In a study of a large number of strains isolated from different geographical areas, four distinct populations were identified (LEHMANN et al., 2006). One population was identified on all continents except South America, two populations were restricted to South America, the fourth population had a ubiquitous distribution. The current distribution of *T. gondii* can be explained by migration from South America to Eurasia and from Europe to North America and Southeast Asia (LEHMANN et al., 2006). KHAN et al. (2007) determined 11 haplogroups in 46 strains of *T gondii*. These haplogroups had a different geographical distribution. This high diversity of *T. gondii* is probably due to host diversity which is much higher in wild host populations (AJZENBERG et al., 2004). Five countries in Europe (Austria, Belgium, France, Slovakia and Slovenia) have recorded active surveillance of congenital cases with mandatory screening of pregnant women. Twenty-two EU/EEA Member States reported toxoplasmosis data to TESSy (21 EU Member States plus Iceland). Denmark, Italy, the Netherlands, Norway, Portugal and Sweden did not have a toxoplasmosis surveillance system. Spain did not have national surveillance and could not provide any estimate for population coverage, so no

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notification rate was calculated. One Member State used the most recent case definition from 2018, eight Member States used the 2012 case definition, nine countries used the 2008 case definition, one Member State reported using the 2002 case definition, two used a different case definition, and did not specify. France regularly reports the highest number of congenital toxoplasmosis cases, most likely due to the sensitivity of its surveillance system that includes screening pregnant women, follow-up of those detected with infestation during pregnancy, and laboratory confirmation of any congenital toxoplasmosis cases detected during the process, including asymptomatic cases (https://www.ecdc.europa.eu/en/toxoplasmosis).

Congenital toxoplasmosis in the EU/EEA increased between 2012-2015, mainly due to reporting by France, which accounted for up to 90% of all cases reported during that period. Even without symptoms, pregnant women can transmit toxoplasma infection to the fetus, which can lead to miscarriage, perinatal death or congenital infection with severe malformation affecting the eyes and brain. Infection in people with low immunity tends to severely affect the central nervous system, but other organs may also be affected. Such patients may require prolonged (sometimes lifelong) therapy. The cost of benefits of prenatal screening programmes have been debated due to the low prevalence of congenital toxoplasmosis in the EU/EEA and uncertainty about the effectiveness of prenatal treatment. A retrospective of the Austrian national prenatal screening programme study concluded that between 1992-2008, it saved social costs of more than €15 million per year and €258 million over 17 years. In France, 79% of maternal infections did not result in clinical symptoms in newborns and birth defects occurred in less than 1%. The authors attributed low morbidity and mortality to early diagnosis and treatment of maternal infections. Nanotechnology is currently being investigated as a tool to manage *T. gondii* infections, as well as the development of vaccines using the mRNA sequence encoding disease-specific antigens. These developments could prove useful in the diagnosis, treatment and possible prevention of congenital toxoplasmosis.

Congenital toxoplasmosis can result in severe disease in infected fetuses. The problem with congenital toxoplasmosis in the EU/EEA is that it cannot be evaluated because of the large differences between national surveillance systems, screening programmes and follow-up of pregnant women. However, regardless of national strategies for surveillance, it is important to strengthen prevention options for congenital toxoplasmosis. Pregnant women at risk of *T. gondii* infection should receive information about exposure and prevention. Based on official reports, more than one billion people are estimated to be infected with *Toxoplasma gondii*, which is transmitted mainly through ingestion of food, water, vegetables and fruit contaminated with sporulated oocysts eliminated by cats or ingestion of vegetative cysts from raw or undercooked meat. The overall cumulative *T. gondii* seroprevalence was estimated at 35% and 59% in domestic and feral cats, respectively, using the random effects model. Seroprevalence was higher in Australia and Africa, where *T. gondii* seropositivity in domestic cats was of 52% and 51% respectively. The lowest seroprevalence was estimated in Asia 27%. The seroprevalence of *T. gondii* in feral cats was of 74% in Africa, 67% in Asia, 67% in Europe and 66% in South America. Infection acquired in the first trimester of pregnancy by untreated women causes congenital infection in 15-25% of newborns. If infection occurs in the second or third trimester of pregnancy, fetal infection occurs in 25-65% of cases. In 70-90% of infected babies, no clinical signs of disease appear, but in a proportion of them clinical lesions manifest as they grow.

The clinical manifestations of the disease may be ocular (chorioretinitis, strabismus, blindness), neuro-psychological (psychomotor or mental retardation, epilepsy, microcephaly, hydrocephalus), haematological (thrombocytopenia, anaemia), other (hypothermia, pneumonia, diarrhoea).

Chronically, in animals the clinical picture is uncertain. In cats, in the case of a low degree of infestation, no clinical symptoms appear. In most cases, in all types of intermediate host infection toxoplasmosis does not manifest by means of clinical signs or is expressed by a mild form of adenomegaly. If infection occurs in pregnant females in whom natural immunosuppression is present, tachyzoites may cross the placenta and contaminate the fetus, leading to fetal death in early pregnancy, miscarriage, birth of a deformed fetus or death by sepsis before 2 months of age.

MATERIAL AND METHOD

The study aimed to determine the prevalence of toxoplasmosis in humans and animals in Iasi County, in order to demonstrate the need for the introduction of monitoring programmes for this protozoa in animals, being a disease underdiagnosed in veterinary medicine in Romania. The study was carried out at the Faculty of Veterinary Medicine in Iasi and at the Praxis medical tests laboratory.

The aim was to determine the prevalence of toxoplasmosis cases registered at the Faculty of

Veterinary Medicine during one year. Since the intestinal form of toxoplasmosis in cats (where oocyst formation occurs - the form of resistance in the environment, and which is the route of contamination of intermediate hosts) is most often asymptomatic or presents mild symptoms, serological diagnosis by detection of antigen and antibodies is recommended.

In the parasitology laboratory the diagnosis of toxoplasmosis is made by detection of antigen and antibodies using rapid veterinary tests - Toxoplasma gondii Ag, Toxoplasma gondii IgG and IgM respectively. WELLTEST *Toxoplasma gondii* Anti is a rapid immunochromatographic test for the qualitative detection of *Toxoplasma gondii* antigens in faecal samples, serum or plasma from animals. It is a rapid assay using the double layer lateral flow immunochromatographic method, sandwich format. Also, to aid in the diagnosis of acute infection it is recommended to follow the antibody titre in the dynamic by assaying for T. gondii specific IgG and IgM titres followed by a second assay 2-4 weeks later.

Maravet Toxoplasma Ab Rapid Test

The Maravet Toxoplasma Ab Rapid Test, which is a qualitative lateral flow immunochromatographic sandwich assay for the determination of Toxoplasma antibodies (CHW Ag) in animal blood, was also used. The principle of the Maravet Toxoplasma Ab Rapid Test is based on the lateral flow sandwich immunochromatographic reaction.

Results of the epidemiological investigations performed at the Praxis laboratory using(*table 1*)

Cats presented to the Parasitology Clinic during 2020 with suspected toxoplasmosis or at a control examination were taken in the study to rule out the presence of the protozoan, and therefore, the risk of contamination of the human host, the owner being pregnant.

RESULTS AND DISCUSSION

Out of a total of 33 cats tested, for the removal of suspected toxoplasmosis, no case was confirmed positive. The majority of the samples evaluated, 26 in number, were from owners who wanted to exclude the presence of *Toxoplasma gondi* in the cat as the owner was pregnant. Suspected cases of toxoplasmosis (4 cases) were those with a digestive clinical picture, mainly diarrhoea with mucus, which did not respond to a usual antiparasitic treatment.

Of the 33 samples evaluated, there were no positive cases. The results of the epidemiological investigations carried out at the Praxis laboratory, Iasi county over a period of 1 year are presented as follows:

Table 1

AB. ANTI *TOXOPLASMA GONDII*- IgM (ELISA) assay/test

Name Age group	Total	Total negative	Total positive	Positive Sex M	Positive Sex F	Total Sex M	Total Sex F
0-12 months	7	7	0	0	0	6	1
1 year	3	3	0	0	0	3	0
2 years	0	0	0	0	0	0	0
3 years	0	0	0	0	0	0	0
4 years	1	1	0	0	0	1	0
5-9 years	1	1	0	0	0	1	0
10-14 years	3	3	0	0	0	1	2
15-19 years	4	3	1	0	1	1	3
20-24 years	21	21	0	0	0	0	21
25-34 years	136	126	10	0	10	1	135
35-44 years	44	40	4	0	4	1	43
45-54 years	3	3	0	0	0	2	1
55-64 years	2	2	0	0	0	1	1
65-74 years	1	1	0	0	0	0	1
75-84 years	0	0	0	0	0	0	0
85+ years	0	0	0	0	0	0	0
TOTAL	226	211	15	0	15	18	208

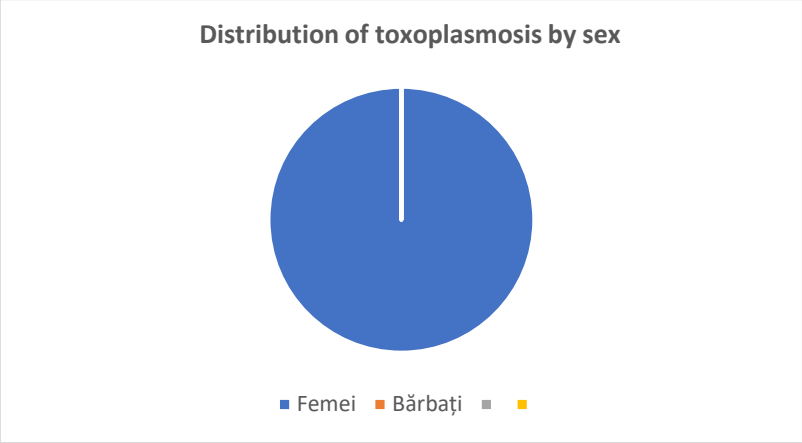

Figure 1 Distribution of toxoplasmosis by sex in human, women-men

As for the distribution of toxoplasmosis by sex, it is very unbalanced, with 100% of cases diagnosed in women. The result is explained by the fact that clinical toxoplasmosis in humans is influenced by the low immune status (*figure 1*). During pregnancy, testing for acute-phase IgM and IgG memory antibodies, respectively, is mandatory, as the disease can cause miscarriage and/or severe congenital malformations in the fetus. The absence of positive cases of toxoplasmosis in the animals studied, but the presence of a considerable number of positive cases in humans, demonstrates that toxoplasmosis is a neglected disease of major importance to human health and is widespread worldwide.

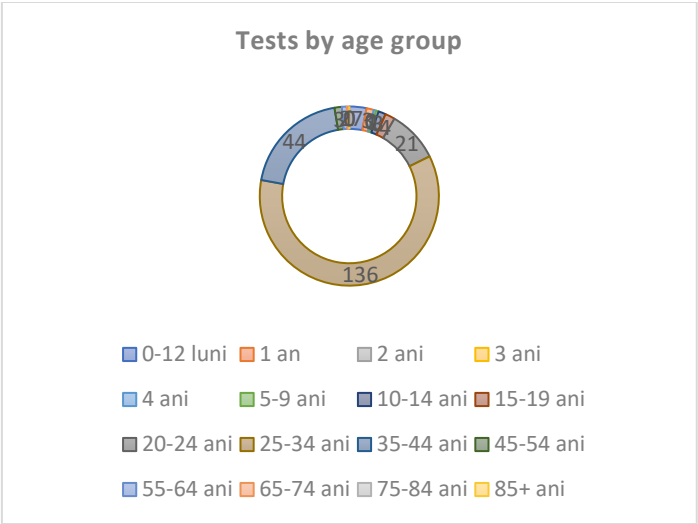

Figure 2 Tests by age group (months/year/years)

Out of a total of 226 tests performed for females and males, 60.17% (136) were performed in the age group 25-34 years, 19.46% (44) were performed in the age group 35-44 years, 9.29% (21) of the tests were performed in the age group 20-24 years, 3.09% (7) of the tests were performed in the age group 0-12 months, and the rest of the tests were performed in the other age groups. The high proportion of testing in the 25-34 age group is strictly related to the number of pregnant women in this age group (*figure 2*).

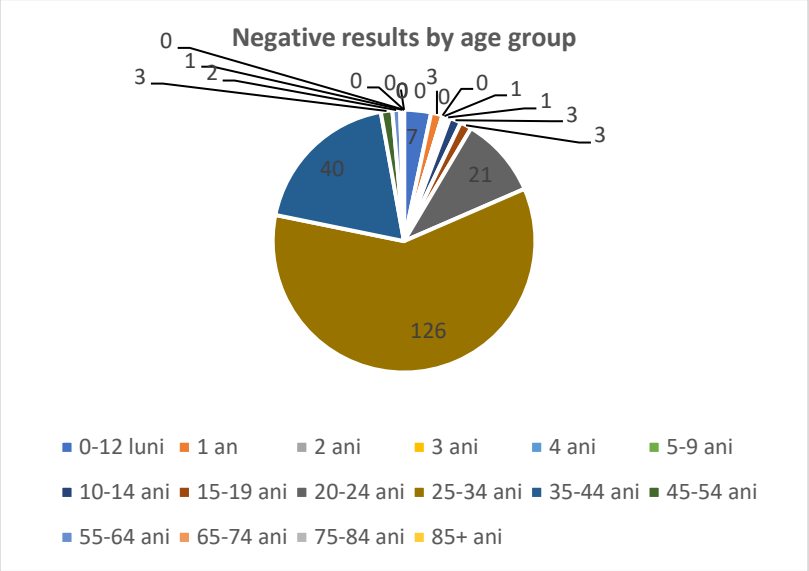

Figure 3 Negative results by age group

Out of a total of 211 negative tests performed for females and males, 59.71% (126) were performed in the age group 25-34 years, 19.95% (40) of the negative tests were performed in the age group 35-44 years, 9.95% of the negative tests were recorded in the age group 20-24 years, 3.31% (7) were performed in the age group 0-12 months, and the rest were performed in the other age groups (*figure 3*).

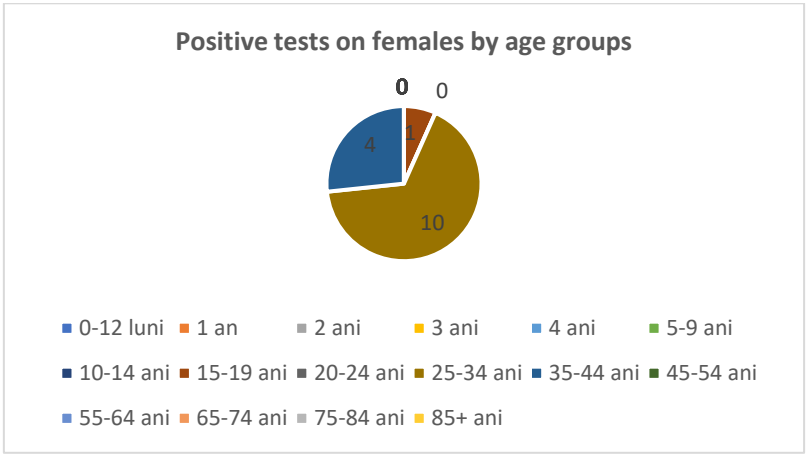

Figure 4 Positive results in females by age group

Of the 15 confirmed cases, 66.66% (10) were in the age group 25-34 years, 26.66% (4) were found in the age group 35-44 years, and 6.66% (1) were found in the age group 15-19 years. The high proportion of positive cases in the age group 25-34 years is directly proportional to the high number of cases tested at this age, being a mandatory test during pregnancy (*figure 4*).

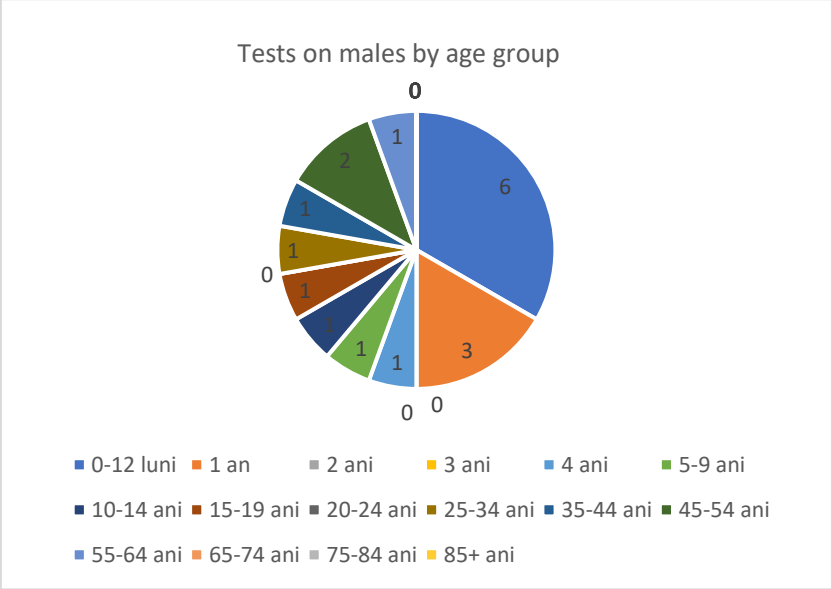

Figure 5 Tests on males by age group

Out of a total of 18 tests performed on males, 6 (33.33%) were performed in the age group 0-12 months, 3 (16.66%) were performed in the age group 1 year, 2 (11.11%) were performed in the age group 45-54 years, and the remaining 7 (38.88%) were performed in other age groups *(figure 5*).

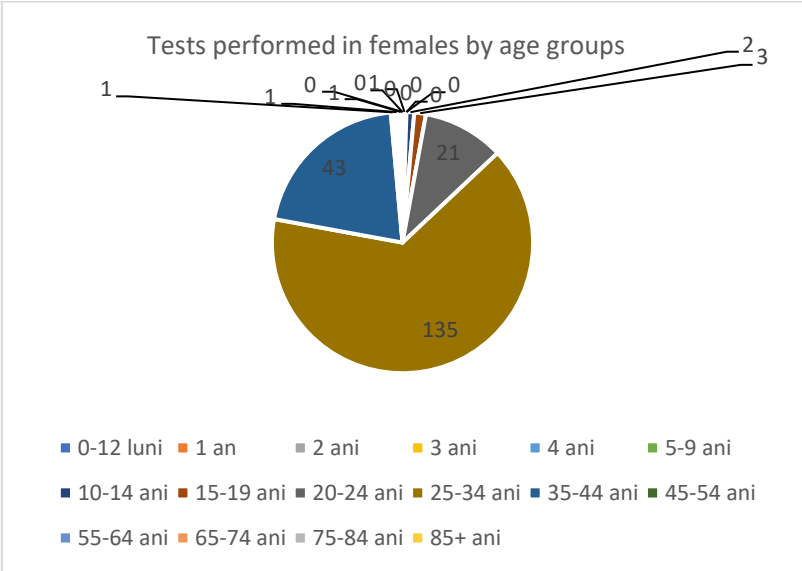

Figure 6 Tests performed on females by age group

Out of a total of 208 tests performed on females, 135 (64.90%) were performed in the age group 25-34 years, 43 (20.67%) were performed in the age group 35-44 years, 21 (10.09%) were performed in the age group 20-24 years, and the remaining 9 (4.32%) were performed in other age groups (*figure 6).*

Results of epidemiological investigations performed at the Praxis laboratory using the AB ANTI TOXOPLASMA GONDII- IgG (ELISA) test*(table 2)*

Table 2

Name Age group	Total	Total negative	Total positive	Positive Sex M	Positive Sex F	Total Sex M	Total Sex F
0-12 months	6	3	3	2	1	5	1
1 year	3	1	2	2	0	3	0
2 years	1	1	0	0	0	1	0
3 years	0	0	0	0	0	0	0
4 years	1	1	0	0	0	1	0
5-9 years	2	2	0	0	0	2	0
10-14 years	2	2	0	0	0	1	1
15-19 years	4	1	3	1	2	1	3
20-24 years	20	18	2	0	2	0	20
25-34 years	134	90	44	0	44	2	132
35-44 years	37	23	14	0	14	2	35
45-54 years	6	4	2	0	2	3	3
55-64 years	3	1	2	0	2	1	2
65-74 years	1	1	0	0	0	0	1
75-84 years	0	0	0	0	0	0	0
85+ years	0	0	0	0	0	0	0
TOTAL	220	148	72	5	67	22	198

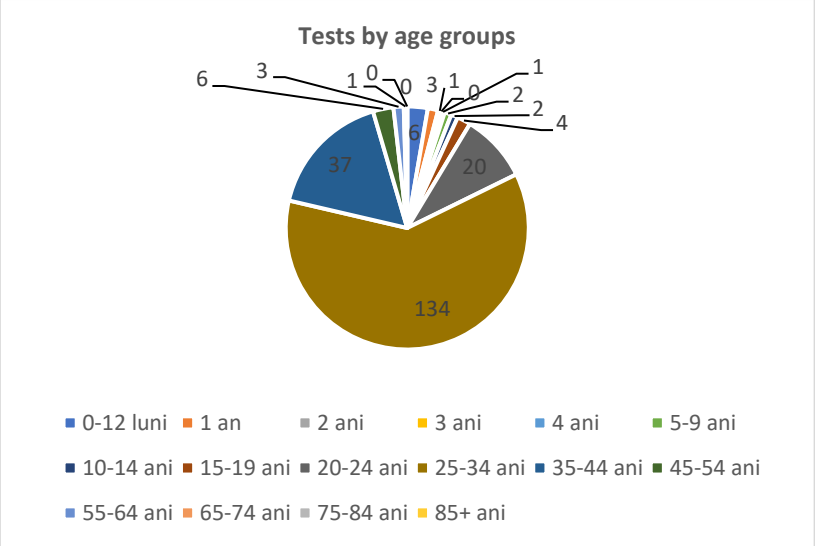

Figure 7 Tests by age groups

Out of a total of 220 tests performed, 134 (60.90%) were made in the age group 25-34 years, 37 (16.81%) were performed in the age group 35-44 years, 20 (9.09%) in the age group 20-24 years and the remaining 29 (13.18%) in other age groups. The majority presence of IgG positive cases in the age group 25-34 years is also directly proportional to the high number of cases tested in this group, closely related to testing during pregnancy (*figure 7).*

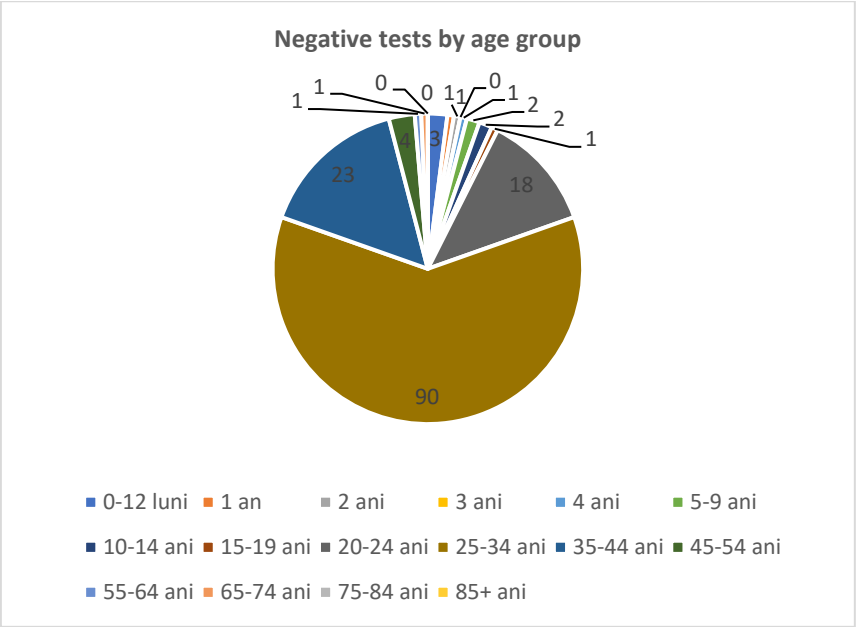

Figure 8 Negative tests by age group

Out of a total of 148 negative tests, 90 (60.81%) were performed in the age group 25-34 years, 23 (15.54%) in the age group 35-44 years, 18 (12.16%) in the age group 20-24 years and the remaining 17 (11.48%) in other age groups. The high prevalence of negative cases in the same age group is influenced by the high number of tests for Toxoplasma at this age(*figure 8*).

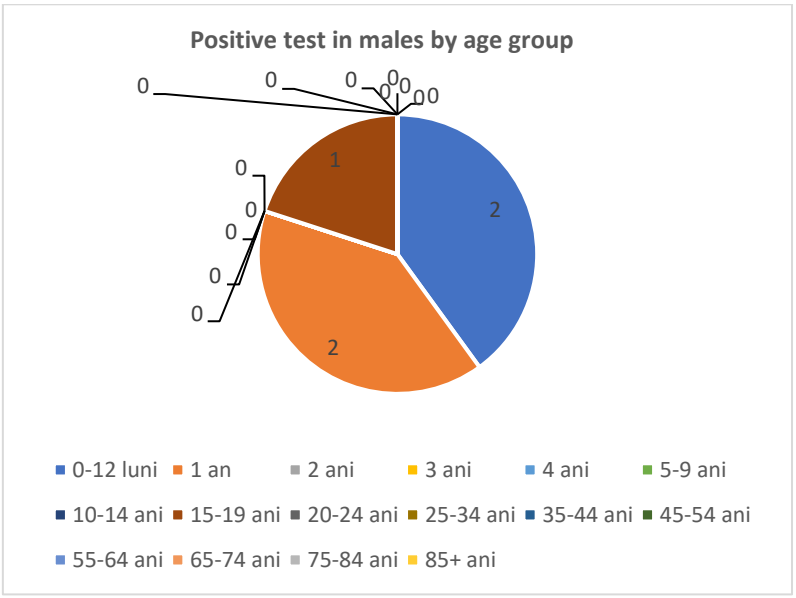

Figure 9 Positive tests in males by age group

Out of a total of 5 confirmed positive tests in males, 2 positive results were recorded in the age groups 0-12 months and 1 year, respectively, and 1 case was confirmed in the age group 15-19 years. Manifestation of the disease in children is related to a deficient immune system under development or to transplacental transmission with manifestation after a variable period of time post birth (confirmed cases in the research literature(*figure 9*).

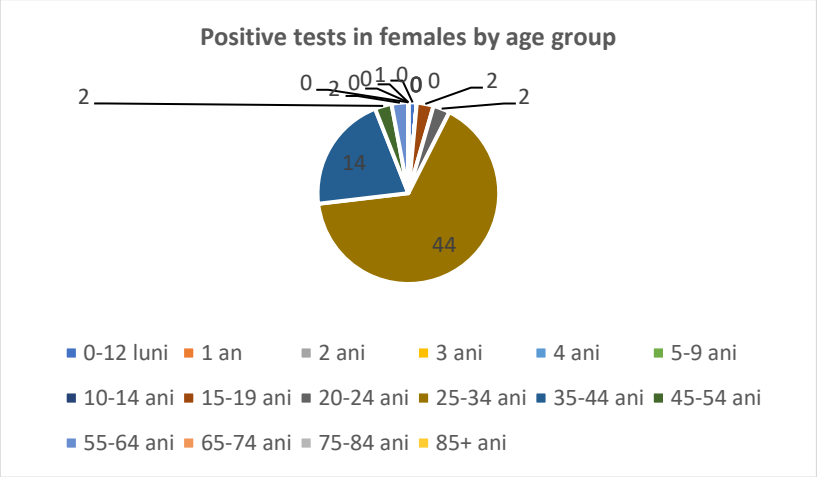

Figure 10 Positive tests in females by age group

Out of a total of 67 confirmed positive cases in females, 44 (65.67%) were in the age group 25-34 years, 14 (20.89%) in the age group 35-44 years, and 9 cases were confirmed in other age groups (*figure 10*).

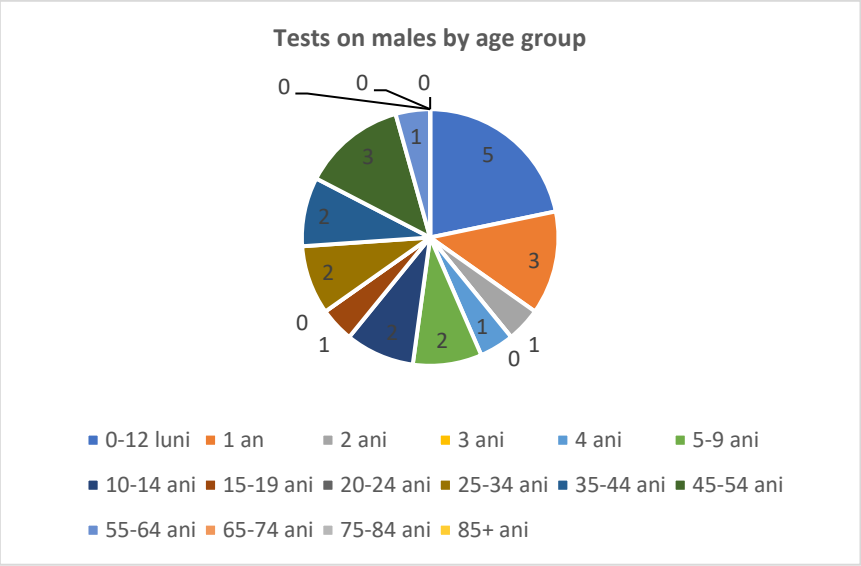

Figure 11 Tests on males by age group

Out of 22 tests performed in males, 5 were performed in the age group 0-12 months, 3 tests performed in the age groups 1 year and 45-54 years, 2 in the age groups 5-9 years, 25-34 years and 35-44 years, and the remaining 5 were performed in other age groups (*figure 11*).

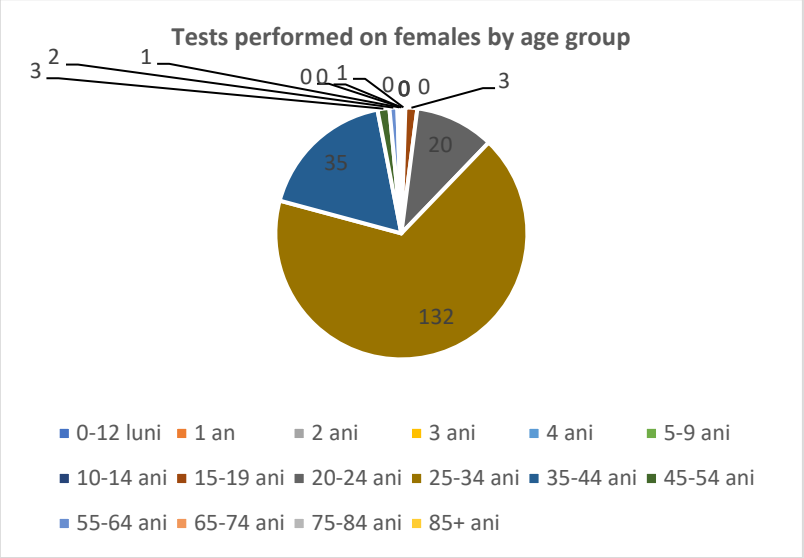

Figure 12 Tests performed on females by age group

Out of a total of 198 tests performed on females, 132 (66.66%) were performed in the age group 25-34 years, 35 (12.62%) in the age group 35-44 years, 20 (10.10%) in the age group 20-24 years and the remaining 11 were performed in other age groups(*figure 12*).

CONCLUSIONS

1. Studies on the spread of toxoplasmosis in animals and humans in Iasi County were carried out at the Faculty of Veterinary Medicine of "Ion Ionescu de la Brad" University of Life Sciences Iasi and at the Praxis medical tests laboratory.

2. Toxoplasmosis is an underdiagnosed protozoa in veterinary medicine due to the non-specific clinical picture, but is relatively well accounted for in human medicine due to the need for testing women during pregnancy. In cats the rapid test for antigen or Ig M is expensive to use in every clinical case suspected of a parasitosis, due to the non-specific clinical picture.

3. Toxoplasmosis is a very serious disease if contracted during pregnancy, causing miscarriage, severe malformations or nerve degeneration manifested even several years after birth. Also, in people with immunosuppressive diseases such as AIDS or autoimmune diseases, toxoplasmic encephalitis occurs, classifying toxoplasmosis as a very serious disease.

4. During 2020, 226 AB. ANTI TOXOPLASMA GONDII- IgM (ELISA) tests were performed in the Praxis laboratory, of which 15 were positive. All positive tests were identified only in females, of which 10 in the age group 25-34 years, 4 in the age group 35-44 years and 1 case in the age group 15-19 years. Most tests were requested by pregnant women during pregnancy.

5. In the Praxis laboratory during 2020, 220 more AB. ANTI TOXOPLASMA GONDII- IgG (ELISA) tests were performed, out of which 72 positive cases were identified, 5 being positive in males in the age groups 0-12 months, 1 year and 15-19 years, and the remaining 67 were identified in females in the following age groups: 0-12 months, 15-19 years, 20-24 years, 25-34 years, 35-44 years, 45-54 years and 55-64 years.

6. The majority of cases were recorded in women in the age group 25-34 years old, showing a much higher testing rate at this age, as this is a mandatory test during pregnancy. This shows that the protozoan *Toxoplasma gondi* is widespread in nature, as toxoplasmosis remains a neglected and under-diagnosed disease in Romania and can be an extremely serious disease.

7. The lack of positive cases in animals during the study, but their presence in large numbers in humans during a single year, shows the major importance of the study for public health, as this very serious disease in pregnant women is underdiagnosed in veterinary medicine.

8. The study is of major importance and warns us about the need to implement serological screening in animals in an attempt to eradicate this disease with serious repercussions for human health, especially in pregnant women and newborn babies.

9. Thus the results obtained can be used as material of high scientific value, in an attempt to educate the population on preventive measures against this disease, which can be contracted through the consumption of fruit, vegetables not properly washed, as well as through the consumption of undercooked meat, being a protozoan with a wide spread in nature.

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