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BRONCHPNEUMONIA AS A HEALTH PROBLEM ON PIG FARMS (RESEARCH REVIEW)

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Summary

Bronchopneumonia is one of the most important respiratory diseases in pigs in intensive breeding. Bronchopneumonia rarely occurs and passes as a monoinfection, and therefore mixed infections are the most common finding. Isolated microorganisms include, for example, Porcine reproductive and respiratory syndrome virus (PRRSV), Porcine circovirus type 2 (PCV-2), *Mycoplasma hyopneumoniae*, Influenza virus, *Actinobacillus pleuropneumoniae, Pasteurella multocida*, *Bordetella bronchiseptica*. Of particular importance in the development of bronchopneumonia in pigs are non-specific factors, such as transport, low temperature, inadequate nutrition, environmental conditions in the boxes, overcrowding and other stressors. Pulmonary pasteurellosis is the result of a lung infection with the bacterium *Pasteurella multocida*. It most often occurs as the last stage of enzootic pneumonia or a complex of respiratory diseases in pigs. The complex of respiratory diseases is one of the most common and economically most expensive diseases of pigs, especially if the pigs come from commercial farms. Pulmonary pasteurellosis is present in different housing conditions of pigs. *P. multocida* as a frequent resident of the nasal flora of pigs is difficult to eradicate since it can interact with many other pathogens. The aim of our study was to examine the antimicrobial susceptibility of bacterial isolates originating from pigs in intensive production.
Keywords: antimicrobial susceptibility, bronchopneumonia, pigs.

Bronchopneumonia or exudative pneumonia is the most common form of pneumonia in pigs and is usually accompanied by cranioventral lung consolidation. Bronchopneumonia can be caused by bacteria, mycoplasmas or food aspiration. Due to the filling of the lungs with exudate, the lungs become hard. According to the quality of exudates, bronchopneumonia can be divided into purulent and fibrinous, although in some cases it is impossible to draw the line between these two types, given that they can be found simultaneously in the lungs or that one form of inflammation can spread to another (18, 19). Purulent bronchopneumonia is characterized by the accumulation of purulent or mucopurulent exudates in the

airways and has a lobular distribution. The inflammatory process in purulent bronchopneumonia is limited to certain lobules of the lungs, which is especially pronounced in pigs. As a result of this distribution of lesions, the lungs affected by purulent bronchopneumonia resemble a chessboard, as a result of mixing color and appearance of unchanged and consolidated lobules. In some cases, abscesses form in the lungs, so this form of purulent bronchopneumonia is referred to as apostematous bronchopneumonia (6, 9). In pathomorphological diagnostics, the appearance of the lungs varies depending on the age of the process. For the first 12 hours, the lungs are swollen and red due to active hyperemia and edema, which are present in the early phase of inflammation. By 48 hours, the lung parenchyma consolidates due to the exudation of neutrophilic granulocytes into the alveoli, bronchioles, and bronchi. The lungs are gray-pink in color and have a harder consistency, and in three to five days they become pale gray, similar to the color of fish meat. Pathohistological diagnosis reveals many neutrophilic granulocytes, macrophages, and desquamated cells in the alveoli, which in extreme cases of purulent bronchopneumonia completely obliterate the lumen of the bronchus, bronchioles, and alveoli (9). *Streptococcus* spp. and numerous species of mycoplasmas, of which *Mycoplasma hyopneumoniae* is the most important for pigs (2, 3, 18).

According to the morphological division, fibrinous pneumonia is exudative pneumonia which is characterized by accumulation of fibrin in the bronchoalveolar spaces. In fibrinous bronchopneumonia, unlike purulent, whole lobes are affected by the changes, which is why fibrinous pneumonia is also called lobar, unlike purulent lobular bronchopneumonia. Fibrinous bronchopneumonia is always preceded by intense damage to blood vessels with appropriate noxa, and this type of bronchopneumonia proceeds through four stages: splenization stage, red hepatization stage, gray hepatization stage, resolution stage (9). In case of unfavorable outcome, there may be a process of organization, which often ends with the growth of parenchyma connective tissue, so the process of fibrinous pleuropneumonia develops in the direction of pulmonary fibrosis, and sometimes fibrous adhesive pleurisy, when adhesions can affect the pericardium (9). One of the complications of fibrinous bronchopneumonia can be necrosis of altered areas of the lung parenchyma, when due to thrombosis of lymphatic and blood vessels, tissue nutrition is prevented and focal foci of coagulation necrosis in the lungs occur, which may be due not only to ischemia but also to pathogenic bacterial toxins (8). The most common causes of fibrinous bronchopneumonia are *Actynobacillus pleuropneumoniae, Pasteurella multocida* and *Mycoplasma hyopneumoniae* (12, 18). Interstitial pneumonia is characterized by an inflammatory process in the interstitium of the lungs. The final diagnosis is made based on pathohistological findings (9). Unlike bronchopneumonia, interstitial pneumonia affects all lung lobes, although in some cases the dorsocaudal distribution of lesions is present. The lungs have a light gray color, mainly due to obliteration of the pulmonary capillaries, and the fleshy or rubbery consistency, without exudates on the cross-section in

uncomplicated cases, originates from cellular infiltration of the pulmonary interstitium. The lungs affected by interstitial pneumonia are rubbery, so they do not collapse after opening the chest cavity, and they have the texture of raw meat on the cross-sectional surface (9). Interstitial pneumonia most often occurs because of aerogenic damage to the alveolar epithelium (toxic gases, free radicals, pneumotropic viruses), but also hematogenous damage to pulmonary capillaries (septicemia, disseminated intravascular coagulopathy, endotheliotropic viruses) or due to local release in the lungs. Based on morphological characteristics, the findings in the acute and chronic course of interstitial pneumonia differ significantly (14, 18). Often, interstitial pneumonia occurs without clear clinical symptoms. As pigs are most often mixed infections (polyinfections), both forms of pneumonia, bronchopneumonia, and interstitial pneumonia, can develop in parallel or can arise from each other. It should be emphasized that interstitial pneumonia is always a precursor to bronchopneumonia. The lungs can respond differently to many agents, and of very different intensity. Morphologically similar changes can be caused by different causes, i.e., the etiologically unique process can go through different stages of morphological changes (9). In all pneumonias, the clinical picture is almost the same. On the other hand, pathomorphological changes may differ in the degree of development of the process and the substrate, which is a consequence of the biological characteristics of the causative agent, virulence and associated action (1, 18).

The aim of our study was to examine the antimicrobial susceptibility of isolates from lung lesions of pigs in intensive production.

Materials and methods

The lung lesions samples were taken under sterile conditions, plated on blood agar plates (blood agar with 5% ram blood) and incubated aerobically at 37°C for 18–24 h. Bacterial isolates were identified using standard methods for phenotypic characterization as previously described (16).

Results and discussions

Many etiological factors participate in the development of respiratory diseases in pigs, with live agents playing the primary role (Table 1, Fig.1). Diseases are rarely caused by a single agent, but they are most often mixed infections (3, 4, 5). The classical division of pathogens into primary (those that are capable of causing the disease themselves) and secondary (those that cause the disease in cooperation with other predisposing factors or pathogens) is becoming less and less acceptable (14). This is due to the fact that a large number of secondary pathogens can cause significant diseases on their own, and on the other hand, a number of isolates or strains of primary pathogens cause very mild or no clinical symptoms of respiratory disorders (6, 18, 19).

The primary causes of respiratory diseases in pigs are PRRSV, swine

influenza virus (Swine Influenza Virus - SIV), swine circovirus type 2 (Porcine Circovirus type 2 - PCV-2, eng), *Mycoplasma hyopneumoniae, Actynobacillus pleuropneumoniae, Bordatella bronchoseptica* in some cases, the virus that causes Aujeszky's disease (Pseudorabies Virus - PRV, eng) and respiratory coronavirus (Porcine Respiratory Coronavirus - PRCV, eng). Among the secondary causative agents, the most important *are Pasteurella multocida, Haemophilus parasuis, Streptococcus suis, Actinobacillus suis, Arcanobacterium pyogenes and Salmonella choleraesuis* (13).

Table 1

Antimicrobial susceptibility of isolated bacteria from pig lungs (%)

	Penicillin	Ampicillin	Amoxicillin	Amoxicillin-Clavulanic acid	Tetracycline	Cephalexin	Gentamicin	Neomycin	Streptomycin	Sulfa Prep.	Enrofloxacin	Florfenicol
Mannheimia	100	100	100	100	100	100	100	100	0	0	100	100
Pasteurella	11	11	11	11	11	22	78	56	0	56	3	89
Streptococcus	100	100	100	100	75	100	0	0	0	75	100	100
Pseudomonas	0	0	0	0	0	0	0	50	0	0	0	100
Haemophilus	100	100	100	100	100	100	100	100	0	100	100	100
E. coli	0	0	0	0	0	100	50	50	0	100	100	100

With the introduction of new diagnostic methods, there is a change in the list of pathogenicity of the causes of respiratory infections. Thus, it has recently been stated that of the infectious agents, a significant role in the etiopathogenesis of respiratory infections belongs to the swine reproductive and respiratory syndrome virus and *M. hyopneumoniae* (12, 18). The special significance of these pathogens is evidenced by the results of conducted research in pig farms in Serbia (20, 21, 22). Characteristically, many of the respiratory pathogens can appear as independent causes, or as often as possible, mutually united in synergistic action. Their prevalence and frequency of occurrence varies, both from west to west, and within the west itself, depending on the technological-production group (17).

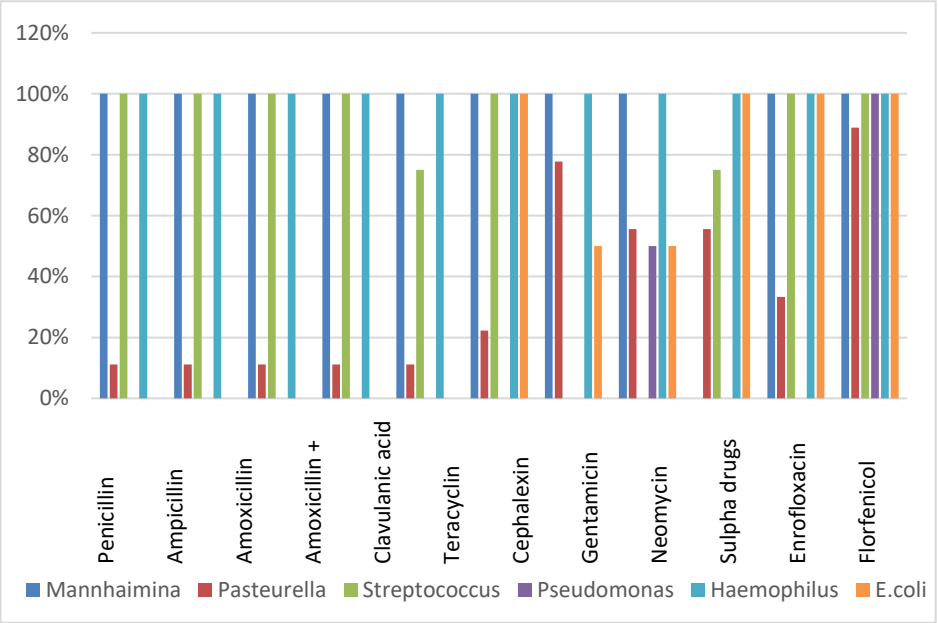

Fig. 1. Antimicrobial susceptibility of isolates

Many of these pathogens can be found simultaneously or sequentially in the same pig farms. In addition to the above, this is the reason that their classical division into primary and secondary respiratory pathogens is not always completely acceptable (7). Some pathogens, previously designated as secondary pathogens, such as *Pasteurella multocida*, can cause the disease on their own and are then considered the primary pathogen (18). It is important to note for diagnostic purposes that the interaction of pathogens is very complex and that each of them independently, in synergism or competition causes a certain manifestation of the disease from the respiratory complex (12). In addition to living agents, special importance is given to non-infectious, i.e., predisposing factors, such as transport, low temperature, bad microclimate, deficient nutrition, overcrowding in box, frequent effects of stress factors. A characteristic of the modern method of production is the formation of agglomerations with a concentration of a large number of individuals in a small space. The consequence is the daily production of large amounts of gases, water vapor, heat and bioaerosol particles, which directly affect the change in physical and chemical composition of the air inside the building, and thus the animal organism (17). In particular, such conditions affect respiratory pathogens and their continuous maintenance of a high degree of virulence in vivo (12). Harmful gases such as ammonia, methyl amine and hydrogen sulfide, which have toxic effects in

high concentrations, also have a noticeable but limited role. It was found that ammonia in experimental conditions, at a concentration of 50 ppm and in production conditions at a concentration of 20 ppm, impairs the function of cilia and thus significantly impairs the defense ability of the mucosal epithelium. Dust, which is usually present in large quantities in pig facilities, has a detrimental effect on the nasopharyngeal and bronchial ciliated epithelium mechanically and chemically. Due to the increased action of predisposing factors, the epithelium of the mucous membrane of the respiratory system is damaged, its activity is reduced, and thus the possibility of continuous elimination of accumulated exudate and inhabited microorganisms (17).

It is inevitable to mention a number of predisposing factors that participate in the occurrence and development of respiratory infections. These are usually significant changes in environmental conditions such as cooling, sudden changes in temperature, increased humidity, overcrowding, poor hygiene, parasitic infections, all of which contribute to the development of the disease. The result is a more frequent occurrence of respiratory infections which are difficult to control, and which are significantly affected by the conditions of keeping in large agglomerations. Technological systems that do not include the "all-in / all-out" procedure, and contain pigs from various localities, introduce gilts into breeding without prior health control and thus form groups of different immune status, thus enabling throat infection in the west by numerous pathogens (15). Changes in environmental conditions lead to stress, which, like various infectious agents, can significantly suppress the respiratory defense mechanisms in pigs. To this should be added the fact that our farms rarely carry out the procedure "rest facility", which would allow minimal exposure to endemic pathogens and thus lead to the development and equalization of the immune status of all individuals in the group (12).

Non-infectious factors can promote the action of mycoplasmas or viruses, which cause primary damage, which is a suitable basis for the settlement and reproduction of other living agents. In this way, a synergistic effect of specific and non-specific factors is achieved, which indicates all the complexity of the etiopathogenesis of diseases of the respiratory system of pigs. At the same time, a very important factor in the pathogenesis of the disease is the susceptibility of the blood vessels of the lungs to the action of numerous immune processes. As a result, damage to the walls of blood vessels occurs, their permeability increases, and consequently edema is created, which is a suitable basis for further pathogenic action of many agents (18).

The harmful effects of etiological factors, as well as their mutual relationship, are constantly being studied. The action of living agents or their toxic products disrupts the defense activity in the lungs, which makes circulation especially difficult, especially in the parenchyma around the edges of the lung lobes. Non-specific factors, such as microclimate and low temperature, are especially important. It has been proven that peripheral cooling can cause disturbance of blood flow through the lungs, with consequent changes in ciliary activity, mucus production, reduction of

local cellular and immune defense activity (18). The pathomorphological changes that occur in respiratory infections are characteristic and depend on the type of infectious noxa, as well as on the ways in which they reach the lungs. The most common route of infection is aerogenic, so that the pathogens reach the lungs through the bronchial tree, where they first spread endobronchially, and then secondarily through the lymphatic pathways to the peribronchial spaces. The pathogens can reach the lung tissue and be hematogenous, especially after septicemic conditions. They first settled in the interalveolar or peribronchial space (11).

There are different views regarding the spread of the causative agent and the occurrence of aerogenic infection. It is believed that nasal secretions, as infectious material, can reach the oral cavity and thus enable the causative agents to first settle in the tonsils and pharyngeal mucosa, and from there reach the respiratory tract (12, 18).

Therapy with antimicrobial drugs is expensive, difficult, and often unsuccessful, the prevention of pneumonia deserves and received more attention. Prevention is usually reduced to changes and improvements in critical breeding points. Techniques in management have been processed by a large number of authors, so attention must be paid to the implementation of their recommendations, because they are recommended on the basis of epidemiological studies, and not from experimental research. They were also done to prevent the occurrence of respiratory diseases and not to differentiate between agents of different etiology. Changes in management can be modification of facilities and reduction of the possibility of spreading microorganisms. Changes in the environment such as increased ventilation, reduced ammonia concentrations, reduced building temperature fluctuations, and reduced dust are some of the most common recommendations. Some of these recommendations cannot be combined, such as increasing the air exchange in winter, resulting in a decrease in the temperature in the building and an increase in humidity, as well as an increase in the amount of dust (10).

Conclusions

Bronchopneumonias of various etiologies cause great damage and problems in pig breeding, and they must be fought against on higher fronts, such as the introduction of new and proven pig breeding technologies, where animals receive complete health care. Prophylaxis should be given as much attention as possible, because only in that way can losses be reduced to a minimum.

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RESEARCH ON RESISTANCE PHENOTYPES OF STAPHYLOCOCCAL STRAINS ISOLATED FROM PETS AND THEIR OWNERS

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Summary

In staphylococcal strains, antibiotic resistance is widespread and the multiple resistant strains are isolated from both, animals and humans, due to the epidemiological circuit of animal-human-animal. Methicillin-resistant staphylococcal strains are extremely important, as resistance to this antibiotic is correlated with multiple resistance to several or all of the used antibiotics. This research aimed to identify the staphylococcal species isolated from pets and their owners, as well as to establish the frequency of their resistance phenotypes. Thus, 45 samples were taken from clinically healthy pets (dogs n = 20, and cats n = 5) that live in close contact with their owners (n = 20). With the Vitek 2 Compact equipment, the isolated strains were identified and included in nine species of *Staphylococcus* genus (2 coagulase positive species and 7 coagulase negative species). The isolates from pets and owners that presented a certain resistance pattern to the used antibiotics had similar frequency of resistance phenotypes, therefore suggesting the possibility of transmitting the resistant strains from animals to humans, and vice versa. However, to confirm the transmission of this resistance, molecular biology tehniques are mandatory, to identify the resistance genes and their transcription.

Keywords: owners, pets, resistance phenotypes, staphylococcal strains.

In bacteria, the antibioresistance represents a very topical problem both in veterinary and human medicine, because it is considered a phenomenon with a pronounced zoonotic risk. Resistance phenotypes of animal pathogenic bacteria, including staphylococci, have an increasing frequency, due to the use of antibiotic-based veterinary medicinal products, particularly in intensively farmed animals (1, 3, 7, 11).

The expansion of multiple antibiotic resistance, in bacterial species pathogenic for animals and humans, has determined the performance of extensive phenotypic and genotypic studies to elucidate this phenomenon, as much as possible. Thus, it has been demonstrated that antibiotic resistance is genetically coded, supported by many resistance genes present in the bacterial chromosome and in mobile genetic elements (plasmid R, intergons, transposons). Through them, genes can be transferred between strains of the same bacterial species (intraspecific transmission), as well as between strains belonging to other bacterial species (interspecific transmission) (1, 2, 7, 11).

In recent years, the resistance phenotypes of staphylococci strains, isolated

from animals, have an increasing frequency and multiple resistance to antibiotics is of particular health importance, because these strains, with a complex epidemiological circuit, can produce, in humans, nosocomial infections (1, 4, 6, 7, 11, 12, 13, 16).

In the last few years, a special attention is paid to methicillin-resistant staphylococci strains, generically known as MRSA (Methicillin Resistant *Staphylococcus aureus*) staphylococci. These strains have a pronounced zoonotic risk and a complex epidemiological circuit, encountered frequently also in humans. Also, the methicillin resistance is associated with multiple antibiotic resistance, especially with resistance to other penicillins, cephalosporins and aztreonam (1, 8, 10, 11, 14, 15, 17).

Data in the literature demonstrate that resistance to methicillin is present in both in coagulase-positive as well as coagulase-negative staphylococci. The extension of methicillin resistance, from strains of *S. aureus* subsp. *aureus* to other species of staphylococci, is generated by the transmission mechanisms of the *mec* staphylococcal chromosomal cassette (SCC*mec*), that exists in the mobile genetic elements of the staphylococcal cytolpasm (1, 2, 7, 11).

This research aimed to identify the staphylococcal species, as well as to establish the frequency of resistance phenotypes of these staphylococci strains, isolated from pets and their owners.

Materials and methods

Samples with pathological material were taken from clinically healthy animals, so that the pathological material contained pathogenic bacteria with etiological significance. These samples were taken from pets (dogs and cats), living in close contact with their owners and, respectively, from their owners. Thus, a total of 45 samples were taken, 20 samples from owners and 25 samples from pets (20 samples from dogs and 5 samples from cats).

In order to isolate the strains of staphylococci, in primary cultures, the samples with pathological material were inoculated in nutrient broth and incubated at 37°C, for 18-20 hours, under aerobic conditions. Next, to obtain the staphylococci in pure culture, inoculations on solid Chapman medium were carried out. Baird-Parker medium was used to emphasize the free coagulase.

For the identification of staphylococcal species, the isolates were subjected to Vitek® 2 Compact equipment analysis. Their identification was done with the efficiency of the Advanced Expert System analysis software, using identification cards for Gram positive bacteria (21).

The strains of staphylococci, isolated in pure culture, were tested for the identification of resistant phenotypes, using antibiotics from the following classes:

- aminoglycosides: gentamicin (CN), tobramycin (TOB);
- *β*-lactams: penicillin G (P), oxacillin (OX), ampicillin (AM), amoxicillin with clavulanic acid (AMC), cefoxitin (CX);
- macrolides: erythromycin (E), clindamycin (CD);

- quinolones: norfloxacin (NX);
- rifamycins: rifampicin (RIF);
- tetracyclines: tetracycline (TE);
- oxazolidinones: linezolid (LZN);
- steroid antibiotics: fusidic acid (FC) (18, 20).

These 14 antibiotics, used to establish the resistance phenotypes, were chosed according to several criteria: the form, respectively the way of administration, the degree of absorption, the therapeutic particularities and effectiveness depending on the species, as well as the correspondence with the therapy of human staphylococcal infections and the emergence of the resistance phenomenon. Also, the choice was made according to the list of antimicrobials of critical importance for human medicine, made by the World Health Organization (The WHO List of Critically Important Antimicrobials for Human Medicine – WHO CIA List) (18).

The resistance profile was made by the disc diffusometric method (KIRBY-BAUER method), using, for this purpose, the Mueller-Hinton medium and biodiscs with the antibiotics mentioned above. Thus, all 43 isolates, included in nine staphylococcal species, were tested.

Testing for the potential relation between the resistance phenotype and the provenience of the staphylococcal strains was performed using the Chi-square test with Yates correction at a level of significance set at $p < 0.05$. Where the requirements for Chi-square testing were unmet, Fischer's exact test was performed with a two-side p value of > 0.05, which is considered significant.

Results and discussions

Analyzing the results, was observed that the positive mannitol staphylococci strains had the highest frequency of 58.14%, and followed by the negative mannitol staphylococci strains and the late mannitol staphylococci strains, whose frequency was 30.23 %, respectively 11.63 %.

Baird-Parker medium was used to identify the coagulase-positive staphylococci strains, respectively the strains that produce free coagulase. Thus, the isolated strains were differentiated in two groups, respectively 7 strains of coagulase-positive staphylococci and 36 coagulase-negative staphylococci strains.

The identification of staphylococci species was made with Vitek® 2 Compact equipment, being subjected to the identification 43 strains, isolated from the owners and their pets (dogs or cats). Thus, seven species of coagulase-negative staphylococci (*S. chromogenes, S. epidermidis, S. haemolyticus, S. lentus, S. simulans, S. vitulinus* and *S. xylosus*) and two species of coagulase-positive staphylococci (*S. aureus* and *S. pseudintermedius*) were identified. The frequency of the isolates was between 2% and 50%, with the highest frequency found in *S. vitulinus* species, followed by *S. haemolyticus* with 21%. Also, was observed that the frequency of coagulase-positive staphylococci species was between 2-14%, while the frequency of coagulase-negative staphylococci species was between 7-50%.

From analyzing the obtained results, it emerged that the number and

frequency of staphylococci species were different, depending on the animal species, respectively the owner from which the samples were taken.

In **dogs**, the samples taken from healthy animals were examined bacteriologically and bacterioscopically and subsequently the isolated strains were identified. Thus, the 18 isolates were included in five species, with a frequency between 11.11% and 33.33% (Fig. 1).

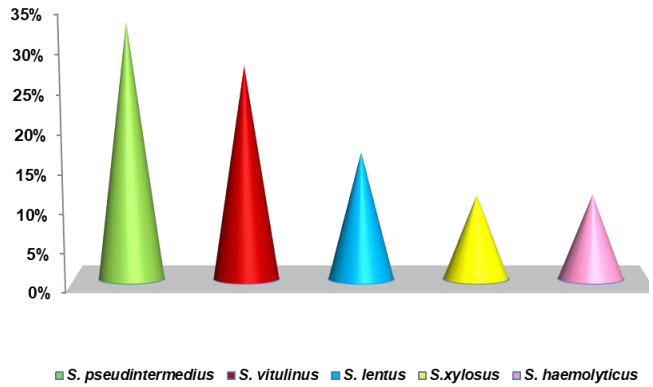

Fig. 1. Staphylococcal species identified in dogs

Therefore, in dogs, the following species of coagulase-negative staphylococci were identifeid: *S. haemolyticus, S. lentus, S. vitulinus* and *S. xylosus*, while from the category of coagulase-positive staphylococci species was identified only one, namely *S. pseudintermedius.* Of the total isolates, the dominant species is *S. pseudintermedius* with a frequency of 33.33%, followed by *S. vitulinus* with a frequency of 27.78%.

The obtained results revealed that, of all the samples taken, in **cats**, were identified only strains of coagulase-negative staphylococci. These strains were included in three species, respectively *S. epidermidis, S. simulans* and *S. vitulinus*. Although a small number of samples were collected, the frequency of ithe solates was different, namely *S. epidermidis* 20%, *S. simulans* 40%, and *S. vitulinus* 40% (Fig. 2).

In cats, compared to dogs, *S. simulans* and *S. vitulinus* coagulase-negative staphylococci species had a higher frequency, suggesting its widespread distribution in various animal species, including pets. Also, the presence of *S. epidermidis* strains suggests an epidemiological circuit between humans and cats.

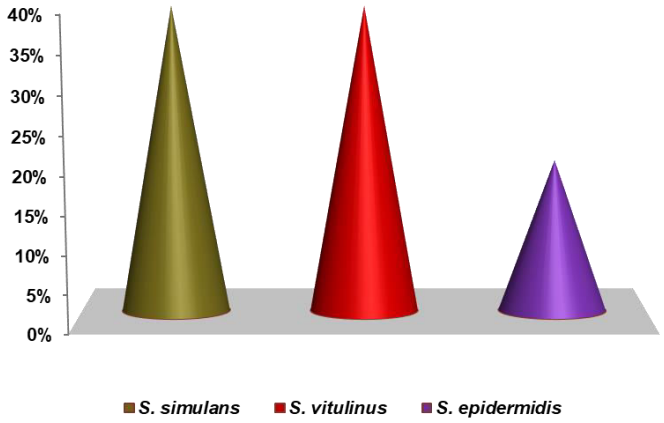

Fig. 2. Staphylococcal species identified in cats

From the **owners** of the pets taken in the study, of all 20 positive samples, different strains of staphylococci were identified, which were included in coagulase-positive staphylococci species (*S. aureus*) and five species of coagulase-negative staphylococci (*S. chromogenes , S. epidermidis, S. haemolyticus, S. simulans, S. vitulinus*). The frequency of these species was between 5%-35% (Fig. 3).

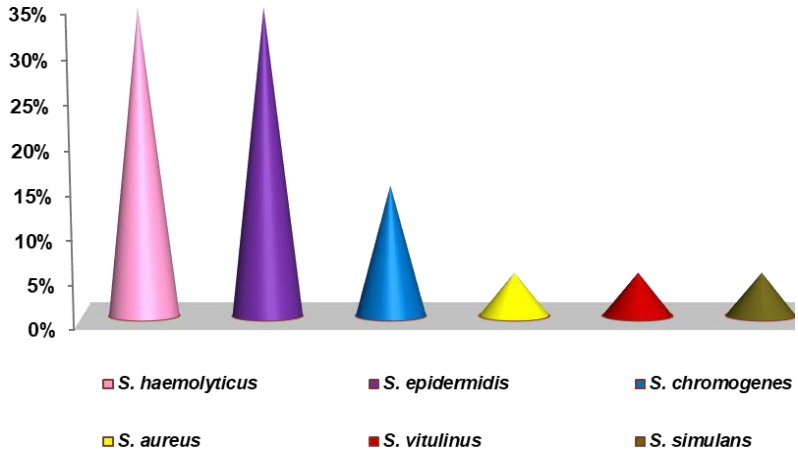

Fig. 3. Staphylococcal species identified in owners

According to the analysis of the data obtained, the species of coagulase-negative staphylococci had the highest values, with the most frequent species *S.*

haemolyticus (35%) and *S. epidermidis* (35%), species that are commensal on the skin and mucous membranes in humans. The presence of the other three species of coagulase-negative staphylococci identified with a lower frequency and which have mostly animals as their natural hosts, suggests that, due to the close contact between humans and their pets, these species can also be found in humans. These aspects were found by other research groups, as well (8, 9, 13, 14, 16).

Furthermore, it is worth mentioning that in five pairs (pet - owner), the identified staphylococci species were the same, namely: *S. haemolyticus* (in two pairs), *S. epidermidis, S. vitulinus* and *S. simulans*, fact that confirms the human-animal epidemiological circuit of staphylococcal species, but also the zoonotic risk for strains like these, which present antibiotic resistance.

The results obtained regarding the identification of resistance phenotypes, depending on the classes of antibiotics, as well as the frequency of susceptibility were processed and shown in Table 1.

Following the obtained results, was observed that the susceptible strains had a frequency between 0% (OX) and 60.47% (LZN), while the resistant strains had a frequency between 39.53% (LZN) and 100% (CN and TOB for *S. aureus* and coagulase-positive staphylococci, OX and CX for *S. epidermidis*). The strains with intermediate susceptibility were found only for four antibiotics (E, CD, RIF and TE), as the interpretations were made according to EUCAST recommendations (19). Thus, for these four antibiotics, the strains with intermediate susceptibility had a frequency between 6.97% (RIF and TE) and 16.28% (E and CD).

The resistance phenotypes were observed for two antibiotics from the **aminoglycoside** group, specifically **gentamicin** and **tobramycin**. According to EUCAST recommendations, for these antibiotics exist two interpretations, depending on the diameter of the inhibition zone, namely one interpretation for coagulase-negative staphylococci strains and one interpretation for *S. aureus*, respectively coagulase-positive staphylococci. Thus, the resistance of coagulase-negative staphylococci strains ranged between 55.56% to CN and 91.66% to TOB, and in the case of coagulase-positive staphylococci strains, the frequency of resistant strains was 100% to both antibiotics. Antibiosensitivity of coagulase-negative staphylococci was between 8.34% for TOB and 44.44% for CN, compared to coagulase-positive strains, where no susceptible strain was identified for these two antibiotics.

From **β-lactams** group, the most antibiotics were selected to identify the resistance phenotypes (penicillin G, oxacillin, ampicillin, amoxicillin/clavulanic acid and cefoxitin) considering that, in the therapy of infections caused by staphylococci, both in animals, as well as in humans, beta-lactams are recommended.

Table 1

Antibiogram results of the isolated staphylococcal strains

Antibiotic	Diameter of inhibition zone (mm)			S		I		R		Tested strains
	S ≥	I	R <	No	%	No	%	No	%	
Gentamicin for *S. aureus*	18	-	18	0	0	-	-	7	100	43
Gentamicin for CN staphylococci	22	-	22	16	44.44	-	-	20	55.56	
Tobramycin for *S. aureus*	18	-	18	0	0	-	-	7	100	43
Tobramycin for CN staphylococci	22	-	22	3	8.34	-	-	33	91.66	
Cefoxitin	22	-	22	6	17.15	-	-	29	82.85	43
Cefoxitin for *S. epidermidis*	25	-	25	0	0	-	-	8	100	
Penicillin G	-	-	R CX	6	13.95	-	-	37	86.05	43
Oxacillin	-	-	R CX	6	13.95	-	-	31	72.09	43
Oxacillin for *S. pseudintermedius*	20	-	20	0	0	-	-	6	100	
Ampicillin	-	-	R CX	6	13.95	-	-	37	86.05	43
Amoxacillin/clavulanic acid	-	-	R CX	6	13.95	-	-	37	86.05	43
Erythromycin	21	18-21	18	12	27.9	7	16.28	24	55.81	43
Clindamycin	22	19-21	19	10	23.26	7	16.28	26	60.46	43
Norfloxacin	17		17	21	48.83	-	-	23	51.17	43
Rifampicin	26	23-26	23	8	18.62	3	6.97	32	74.41	43
Tetracycline	22	19-22	19	9	20.94	3	6.97	31	72.09	43
Linezolid	21	-	21	26	60.47	-	-	17	39.53	43
Fusidic acid	24	-	24	16	37.2	-	-	27	62.8	43

Legend: C = concentration; CN = coagulase negative S = sensitive strains; I = strains with intermediate sensitivity; R = resistant strains; R CX = strains resistant to cefoxitin are resistant to all β-lactams (19)

According to EUCAST recommendations, for the identification of methicillin-resistant strains, that have a pronounced zoonotic character and that can be transmitted both from animals to humans and vice versa, the cefoxitin-resistant strains are resistant to all antibiotics in this group. Thus, in case of **cefoxitin**, depending on the diameter of the inhibition zone, there are two interpretations, namely one interpretation for the *S. epidermis* species and another interpretation for

the other staphylococci species (19). According to these indications, the results obtained showed that in the 8 isolates of *S. epidermis*, the frequency of resistant strains was 100%, while in the case of the other species of staphylococci, the frequency of resistant strains was 82.85%, while the antibiosensitivity had a frequency of only 17.15%. Therefore, for the other antibiotics in this group (**ampicillin**, **amoxicillin with clavulanic acid** and **penicillin G**) the antibiosensitivity and antibioresistance were reported according to the results identified for cefoxitin.

For **oxacillin**, depending on the diameter of the0 inhibition zone and the staphylococcal species, there are also two interpretations: an interpretation for the *S. pseudintermedius* species (for the screening of methicillin-resistant strains) and an interpretation for the other species, which is in concordance with the results obtained for cefoxitin (19). Therefore, analyzing the results obtained, the resistant strains of *S. pseudintermedius* species had a frequency of 100%, while for the other species antibiotic resistance was present in a percentage of 72.09%.

Two antibiotics were selected from the **macrolide** group, namely **erythromycin** (that indicates the inducible resistance to 14-atom macrolides) and **clindamycin** (which indicates the inducible resistance to 16-atom macrolides). Of all 43 strains, the resistant strains had a frequency of 60.46% for clindamycin, respectively 55.81% for erythromycin. Within this group, strains with intermediate susceptibility were also identified, with a frequency of 16.28% to both antibiotics. The frequency of isolated staphylococci strains susceptibility to erythromycin was 27.9%, followed by the frequency of staphylococci susceptibility to clindamycin, (23.26%).

Resistance phenotypes to **quinolone** group were observed only for **norfloxacin**, an antimicrobial of critical importance (with the highest priority), according to the classification made by the WHO (18). Hence, analyzing the results, was found that the frequency of resistant strains (51.17%) was slightly higher than that of susceptible strains (48.83%).

From the group of **rifamycins**, resistance phenotypes were monitored for **rifampicin** antibiotic, also a critically important (high priority) antimicrobial, according to WHO classification (18). For this antibiotic, the frequency of resistant strains was higher (74.41%), compared to the proportion of susceptible strains (18.62%) and that of strains with intermediate susceptibility (6.97%).

The resistance phenotypes to antibiotics from **tetracycline** group were established using only **tetracycline**, because basic tetracycline or oxytetracycline are most often used in animal therapy, but also in humans, because is an antimicrobial of great importance in human therapy (18). Consequently, the results showed a frequency of 72.09% for the resistant strains, considerably higher than the one found for susceptible strains, which was 20.94%, or that of strains with intermediate susceptibility (6.97%).

Antibioresistance to **oxazolidinone** antibiotics was monitored using **linezolid**, a strictly human antimicrobial of critical importance according to the WHO list (18). After analyzing the results, a frequency of susceptible strains of 60.47% was obtained, higher than the one of resistant strains, which was 39.53%.

Another highly important antimicrobial used in human therapy, that was selected was **fusidic acid**, which belongs to the group of **steroid antibiotics** (18). However, for this antibiotic, unlike linezolid, the frequency of resistant strains (62.8%) was higher compared to that of susceptible strains (37.2%), which could indicate an interspecific transmission of the resistance phenomenon.

In this research, another objective was the identification of resistance phenotypes to the tested antibiotics, of the isolates, depending on their origin, respectively from pets or their owners (Table 2).

Table 2

Resistance phenotypes of staphylococcal strains isolated from pets and their owners

	Owners		Pets		Statistical analysis	
	20 strains		23 strains			
	No	%	*No*	%	X^2 value	*p*
Gentamicin	13	65	15	65.2	0.093	0.75
Tobramycin	19	95	21	91.3		1
Cefoxitin	15	75	22	95.7		0.08
Penicillin G	15	75	22	95.7		0.08
Oxacillin	15	75	22	95.7		0.08
Ampicillin	15	75	22	95.7		0.08
Amoxicillin/Clavulanic acid	15	75	22	95.7		0.08
Erythromycin	13	65	18	78.3	0.39	0.53
Clindamycin	14	70	19	82.6		0.47
Norfloxacin	10	50	12	52.2	0.02	0.87
Rifampicin	16	80	19	82.6		1
Tetracycline	15	75	19	82.6		0.71
Linezolid	7	35	9	39.1	0.0014	0.97
Fusidic acid	7	35	17	73.9	5.09	0.02*

A number of 23 strains of staphylococci were isolated from **pets**, namely dogs and cats, which had the following characteristics to the tested antibiotics: the frequency of susceptible strains was between 4.34%-60.87% and the frequency of resistant strains was between 95.66%-4.34%. In the case of those four antibiotics, which also had an interpretation for intermediate susceptibility, the strains isolated in pets had a frequency between 4.34% and 26.09%.

From the **owners**, a total number of 20 strains of staphylococci were isolated, which behaved differently to the antibiotics used for testing: the frequency of susceptible strains was between 5%-65%, while the resistant strains had a

frequency between 5%-90% and strains with intermediate susceptibility identified for four antibiotics had a frequency between 5% and 10%.

Analyzing the results obtained regarding the resistance phenotypes to selected antibiotics, identified in pets and their owners, some similar frequencies were observed between the two categories. Thus, with the exception of fusidic acid, associations were found between the resistance phenotypes and the origin of the strains ($p > 0.05$). The prevalence of resistance to the tested antibiotics of the strains isolated from owners and their pets was similar to gentamicin, tobramycin, clindamycin, norfloxacin, but also to fusidic acid, the results indicating the possibility of spreading these strains of multi-resistant staphylococci due to closely contact between pets and people.

The frequency of fusidic acid resistance phenotype was significantly higher in strains isolated from pets (73.9%) compared to strains isolated from owners (35%) X^2 (1, N = 43) = 5.09, p = 0.02.

The obtained results revealed the presence of resistance phenotypes to coagulase-positive and coagulase-negative staphylococci strains isolated from both pets and their owners. Multi-resistant strains of staphylococci are increasing in frequency and the identification of resistance phenotypes to antibiotics used in therapy suggests the continued expansion of this phenomenon through a complex two-way animal-human epidemiological circuit. Hence, there are numerous researchers that focus on the identification of resistant strains of staphylococci and their transmission from animals to humans and vice versa (4, 5, 6, 13, 15, 16, 17).

In 2020, in Saudi Arabia, Hemeg et al. (8) found that pets carry various microorganisms with serious public health risks for humans. Therefore, they investigated pets, mainly dogs and cats, as a reservoir for MRSA and the genetic similarity between MRSA strains collected from pets and their owners. The prevalence of MRSA was higher in dog swabs than human swabs. Dog swabs showed a frequency of 44.4%, in cats 27.3%, while in owners' swabs a frequency of 42.8% was detected. Antibiotic resistance profiles had a frequency of 69.2% and all MRSA strains were positive for the *mec*A gene (100%). The authors results showed that there is a high similarity between the strains, indicating that pets play an important role in the colonization and transmission of MRSA to humans and vice versa (8).

Also in 2020, in South Korea, Oh et al. (10) investigated the distribution and epidemiological link of methicillin-resistant *S. aureus* (MRSA) isolates from companion dogs, owners and residential environments of 72 households. Antimicrobial susceptibility testing and PVL gene determination was performed using PCR. A total of 65 *S. aureus* strains (2.5%) were isolated and 49 (1.9%) of 65 strains were MRSA positive, with resistance to cefoxitin also present. Strains were isolated from dogs (9.2%), owners (41.5%) and residential environments (24.6%). ST72-SCC*mec* IVc MRSA clones appeared predominantly in MRSA-positive familie. Additionally, PFGE analyzes showed that ST72 SCC*mec* IVc-t324 were also found in owners and dogs and the authors concluded that this study is among the first to report the exchange of MRSA ST72 between dogs and their owners (10).

Therefore, the results obtained, regarding the resistance phenotypes of staphylococcal strains, underline the importance of identifying these strains, which have a pronounced zoonotic character, with pets acting as a true microbial reservoir for humans, especially pet owners.

Conclusions

With the use of Vitek 2 Compact, the isolated strains were identified and included in two coagulase-positive and seven coagulase-negative staphylococcal species.

The strains of staphylococci isolated from pets had maximum resistance to *β*-lactams, followed by tobramycin, and maximum susceptibility to linezolid.

The staphylococcal strains isolated from the owners had a maximum susceptibility to linezolid and fusidic acid and a maximum resistance to tobramycin, followed by *β*-lactams.

The isolates from pets and owners that presented a certain resistance pattern to the antibiotics used had similar frequency of resistance phenotypes, therefore suggesting the possibility of transmitting the resistant strains from animals to humans and vice versa.

However, molecular biology tests, which certify the presence of resistance genes and their transcription, are mandatory to confirm the transmission of these resistant strains.

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PRELIMINARY RESEARCH REGARDING THE PREVALENCE OF CATTLE NEOSPOROSIS IN CARAS-SEVERIN COUNTY

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Summary

Because neosporosis is less studied in Romania and it implies severe economic loses and because of the difficulties in establishing the diagnosis of infection with *Neospora caninum* in farms we proposed to perform a serological screening in cows in Caraş-Severin County to determine the prevalence of this protozoan. During the period June 2020 – April 2021, 110 blood samples from bovines reared in the household system were collected. After expression of the serum, it was collected in Eppendorf tubes, labelled and frozen at -18°C until ELISA processing to highlight the infection with *N. caninum*. The ID Screen® *Neospora caninum* Indirect kit from ID Vet France was used. Out of the total of the 110 investigated samples only 15 were positive, which represents 13.51% of the samples corresponding to Caraş-Severin County. The serologically examined cattle came from 19 localities, most of them being located in the Almăj area. Of the 19 localities investigated, just over 50% were positive.

Keywords: neosporosis, cattle, Caraș-Severin County, ELISA.

Neosporosis is a bovine protozoosis which cause important economical loses due to abortions, elimination of cows with repeated abortions, re-inseminations, possible loss of lactation, costs related to diagnosis and professional assistance. Affected calves show neurological signs, they are weak, unable to stand up or sometimes they are born without clinical signs (1, 5, 6, 9, 14, 18, 20).

In the parasite life cycle the dog is definitive host. In dogs the parasite develops in the digestive tract, while in intermediate hosts it develops systemic, mainly in the central nervous system, in skeletal and cardiac muscle tissues. The intermediate hosts' infection took place by ingestion of sporulated oocysts with water or food, but the most important contamination route remains the vertical one, namely transplacentally (5, 6, 11, 12, 17, 19).

Neospora, as well as other protozoans (*Sarcocystis* spp*., Cryptosporidium* spp.), can be also found in a wide range of both domestic and wild animals (3, 4, 7, 8, 10). Together with dogs, coyotes or red foxes could act as definitive hosts and roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*) and red deer (*Cervus elaphus*) as intermediate hosts (2, 3, 4, 15).

The aim of the present study was to assess the prevalence of *N. caninum* in a cohort of cattle from Caraș-Severin County, Romania

Materials and methods

During the period June 2020 – April 2021, 110 blood samples were collected from cows traditionally reared in Caraș-Severin County.

Sampling was performed from the jugular vein, in sterile vacutainers without anticoagulant.

After expression of the serum, it was collected in Eppendorf tubes, labelled and frozen at -18°C until the time of processing by ELISA, to highlight the infestation with *Neospora caninum*.

The ID Screen® *Neospora caninum* Indirect kit from ID.Vet, France, was used, according to the manufacturer indications.

Fig. 1. The ID Screen® *Neospora caninum* Indirect kit (left) and the incubated ELISA microplate (right)

For each sample the ratio S/P (S/P%) was calculated according to the formula:

$$S/P\% = \frac{DO_{probă} - DO_{MN}}{DO_{MP} - DO_{MN}} \times 100$$

where:
*DO = optic density
*DO_{MN} = optic density negative sample
*DO_{MP} = optic density positive sample

Results and discussions

Out of the 110 investigated samples, only 15 were positive, representing 13.51% from all samples corresponding to Caraș-Severin County.

The examined bovine belonged to 19 villages and the majority of the sampled cattle came from Almăj area.

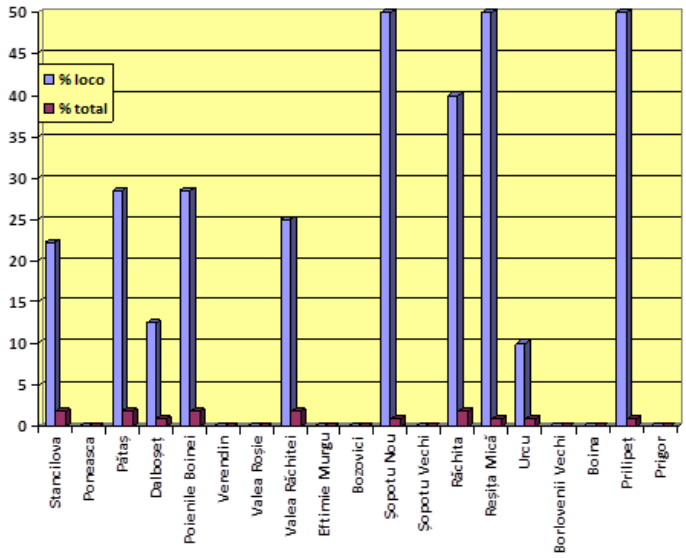

Fig. 2. Local and general prevalence in investigated localities.

In 9 localities from all 19 examined (47.36%) negative animals were identified. However, the total prevalence on each locality never exceeds 1.81%, the most affected being: Stancilova, Pătaș, Poienile Boinei, Valea Răchitei and Răchita. Also, the most numerous positive cattle out of 15 were noticed in Șopotu Nou village (10), representing 66.67%.

In Europe, the prevalence was different from county to country. Thus, in Netherlands, prevalence varied between 9.9% and 39.4% and in Italy was 18.8% in the southern and 30.8% in the northern of the country, respectively. For Poland, the maximal value was 15.6%, in Slovakia was 22.2%, in Sweden fluctuated between 1.3% and 65%, in Turkey was obtained a lower range of values between 0% and 13.9%, while in France the limits were 5.6% and 26% (12).

In Romania, data regarding *Neospora caninum* infection are recent but scarce, first study being conducted by Ionescu et al. (17). Data provided by Gavrea et al. (13) shown a low prevalence in cattle from households (0%-16%), with an

average of 5.4%, but a very high prevalence in cattle from intensive systems (60.3%). The values reported in the households was similar to those obtained by us in Caraș-Severin County. The study carried out by Imre et al. (16) in three counties from western Romania (Bihor, Arad and Timiș) on 376 serum samples obtained from cattle from 25 farms revealed an overall prevalence of 27.7%, without significant fluctuations inside of each county (26.9%, 27.9% and 27.9%, respectively).

Conclusions

Serological investigations performed on cattle from households in Caraş-Severin County showed a prevalence of 13.51% for *Neospora caninum* infestation.

Most positive cases were registered in the villages belonging to Șopotu Nou commune, in the Almăjului Mountains area.

Of the 19 localities investigated, just over 50% were positive.

Considering the fact that most of the samples came from cattle from the south of the county, near the Almajului Mountains, where the possibility of contact with the final hosts in the sylvatic cycle is high and the prevalence indicated on positive localities is relatively high, other studies are needed to cover the entire area of the county.

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DETERMINATION OF METHOTREXATE PHARMACOKINETICS BY SCINTIGRAPHIC MONITORING OF 2,4 DIAMINO 6-PERTECHNETATE BIODISTRIBUTION

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Summary

The use of methotrexate (MTX) as a photosensitizer in combination with optical irradiation of solid malignancies initially requires determination of the intratumoral retention time of the cytotoxic agent, at the time of administration. This can be done with the help of sequential scintigraphy and, implicitly, involves the radioactive marking of MTX with the 99mTc isotope. Radioactive chromatography (RC) and UV-VIS absorption spectroscopy confirm the possibility of synthesizing a radiopharmaceutical (2,4-diamino 6-pertechnetate pterin) that follows the MTX biodistribution. In addition, from the first experimental data performed on animals (Wistar rats with Walker 256 solid tumors), it indicates a therapeutic index of this cytostatic-radioactive compound, even in the absence of the Photostimulated Chemotherapy protocol.

Keywords: Methotrexate, Photoensitizer, Radioactive marking, Radiochromatography, Scintigraphy.

It is to be expected that 14 million of people develop cancer each year, and this number could increase to more than 21 million until 2030. This disease is responsible for almost one in each six deaths worldwide. Each year, 8.8 million of people died from cancer, especially in low-income countries (1). Among the causes of death by cancer is cachexia, responsible by 20% of death. This complication in oncologic patients cooperate to a worse prognostic, lower survival, alterations quality of life, deterioration in functional capability, as well as significantly contribute to toxicity induced by chemotherapy (2, 3). About 30% of human patients diagnosed with breast cancer develop metastatic brain tumors, making them very difficult to investigate and treat due to the specific nature of this organ (7, 9, 16). However the animal models are important tools to study this disease and develop drug therapies (10, 12, 17).

MTX absorption and fluorescence spectroscopy was performed on irradiated natural saline solutions in the near UV range, using a mercury vapor lamp. Nonlinear changes were detected depending on the exposure time which ranged from 3 to 180 minutes (6). Spectral changes indicated photodissociation of MTX into 2,4-diamino-6-formylpterin and p-amino-benzyl-glutamic acid compounds (9, 11). As in the similar

case of folic acid photodissociation, the generation of highly reactive singlet oxygen molecules (1O2) was observed and were detected during the reaction (6, 8).

Materials and methods

Synthesis of the radiopharmaceutical product

The technetium generator (activity of 12 GBq) was obtained from the Institute of Isotopes Co. Ltd. (Budapest, HU). The actual synthesis of the radiopharmaceutical consisted in mixing the lyophilized powder of Methotrexate, purchased from SINDAN S.R.L. (Bucharest, RO) with stannous chloride in a ratio of 1/10, in ascorbic acid medium. The process took place in a sealed container which was subsequently vacuumed. Then, sodium pertechnetate eluate (500 μCi (18.5 MBq) activity, measured with a MicroCal dose calibrator - PICKER, USA) was added to the container.

Highlighting the radioactive marking

It was performed by gel chromatography. Sephadex G-25 fine gel purchased from Pharmacia Uppsala (Sweden) was used for gel filtration. A chromatographic column with an internal diameter of 9 mm and a length of 160 mm was packed. The fixed parameters of the procedure that remained constant throughout the determinations were: eluent flow rate (0.9% natural saline) = 2 ml/min; fraction acquisition time = 30 sec; number of fractions collected/determination = 80.

The 80 fractions collected were measured for activity using a Gamma 5500 gamma counter (Beckman, USA). The measurement time was 60 sec / fraction.

Initially, the “peak” radioactive fractions corresponding to the vacuum volume and sodium pertehnetate were identified using radiolabeled Blue Dextran 2000 (Blue Dextran 2000 was purchased from Pharmacia Uppsala, Sweden). Subsequently, analyzing the previously prepared radiopharmaceutical, the corresponding peak radioactive fraction was also identified. Radioactive labeling certainly occurred because all three peak radioactive fractions (corresponding to vacuum volume, pure sodium pertennetate, and radiopharmaceutical) differ in the collection sequence beyond the margins of error, keeping acquisition parameters constant during both determinations (19, 23).

Identification of the marked compound

A Secomam S-750 UV-VIS spectrophotometer, France, was used for this purpose. The absorption spectrum (200 - 900 nm) of the corresponding "peak" radioactive fraction was purchased. The presence of the pterinin nucleus in the structure of the marked compound was determined by comparison with the 6-formylpterin spectrum (24, 26).

Experimental animals

Three ten-week-old male *Wistar* rats obtained from the Institute of Oncology, Bucharest, Romania were used. The animals were inoculated with Walker 256 solid tumors, at various locations. The "in vivo" experiments were performed in accordance with the Ethics Council of the Institute of Oncology, Bucharest (13, 20).

Scintigraphic biodistribution studies

The laboratory animals were injected into the caudal vein with the radiopharmaceutical obtained and then sedated with ethyl ether. Immediately after i.v.,100 µCi of the previously obtained compound was injected (15). The scintigraphic images were acquired successively at an interval of 15 minutes, for one hour, using an Anger camera type DX 2000 SPECT produced by PICKER, USA (thyroid mode was used). The activity administered to each rat was measured using a MicroCal dose calibrator (PICKER, USA). No sacrifices were made at the end of the experiments.

Results and discussions

Preparation of the radiopharmaceutical product:

Upon addition of sodium pertechnetate eluate (Tc04Na) with an activity of 18.5 MBq in the vacuum flask containing the mixture of MTX-SnCl2 and traces of ascorbic acid, a slight precipitation was observed. Therefore, the solution was centrifuged for 3 minutes at a speed of 2000 rpm. The ratio between the activity of the precipitate (0.6 MBq) and the supernatant (17.8 MBq) indicates that a radioactive marking process has taken place.

Highlighting the radioactive marking:

The procedure consisted in applying the sample (50 µl) Blue Dextran 2000 solution (marked with 99 m Tc) in the chromatographic column and starting the gel filtration. The solution was obtained by diluting Blue Dextran 2000 (powder) in sodium pertechnetate eluate in the presence of traces of ascorbic acid (14). After collecting and measuring the activity of the 80 fractions, the elution curve shows the appearance of two peaks centered on fractions 12 and 46 (in the order of collection). The two peaks correspond to Blue Dextran 2000 (vacuum volume) - channel 12 and sodium pertechnetate - channel 46 (Fig. 1). Keeping the operating parameters of the chromatographic analysis unchanged, an identical amount (50 µl) of prepared radiopharmaceutical was applied and fractionated. After measuring the radioactivity of the fractions, the appearance of a peak centered on channel 22 was observed (Fig. 2). The difference between the elution curves corresponding to the vacuum volume (radioactive fraction of channel 12), radiopharmaceutical (radioactive fraction of channel 22) and sodium pertechnetate (peak radioactive fraction 46) attests to the production of a radioactive marking.

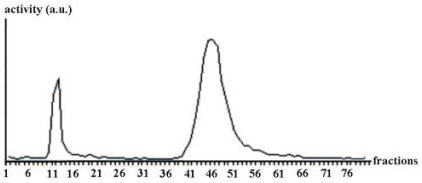

Fig. 1. Elution curves obtained corresponding to sodium pertechnetate and Blue Dextran 2000 marked

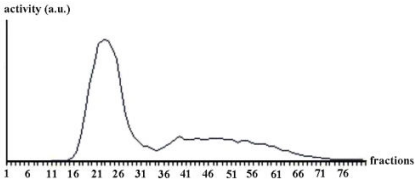

Fig. 2. Radiopharmaceutical's elution curve (marked compound)

Identification of the marked compound:

Acquisition of the UV-VIS absorption spectrum of fraction 22 (obtained by gel filtration of the radiopharmaceutical) in the range of 200-900 nm reveals the existence of a single strong absorption band centered on the 285 nm wavelength (Fig. 3).

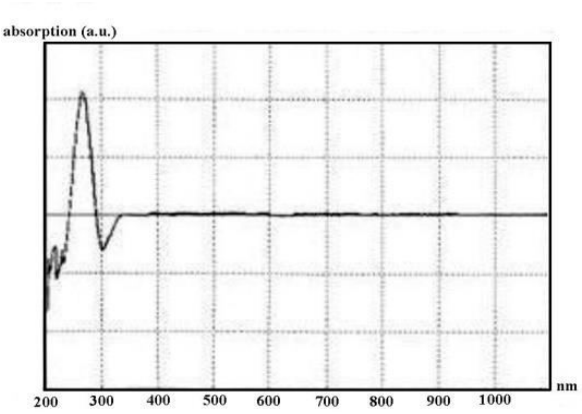

Fig. 3. Absorption spectrum of the radioactive sample

This unique absorption band is distinctive for pterin compounds and was also highlighted - associated with the photoproduct 6-formylpterin - during photolysis of folic acid (8, 9). Thus, it is very likely that the radiopharmaceutical obtained is in fact a 2.4 diamino 6-pertechnetate pterin.

Scintigraphic biodistribution studies: (animal experiment)

After the i.v. administration of the radiopharmaceutical in the caudal vein (0.5ml / 3.7 MBq) scintigraphic images were purchased at a frequency of 15 min. Thus, four distinct stages of the "in vivo" biodistribution of the radiopharmaceutical were highlighted s follows:

- In a short interval from the “bolus” administration of the radiotracer (less than 15 min), it accumulates in the liver where a part is metabolized by hydrolysis;
- After half an hour a double “migration” of the substance is observed: the metabolized fraction is conducted in the bladder to be eliminated, and the “active” (antineoplastic) fraction is conducted in the tumor area (the active substance fraction is visibly higher than the metabolized one);
- At an interval of approx. 45 minutes after administration, the actual fixation of the agent in the tumor mass begins;
- One hour after administration, it can be said that the tumor hyperfixation reaches a peak (18, 22, 26).

We also noticed that the initial retention of the substance in the liver (not in the thyroid and bladder) undoubtedly indicates the production of radioactive marking of the pteridine nucleus in the MTX molecule. For example, in fig. 4, four scintigraphic sequences of an animal with a left axillary primary tumor are shown. Images were purchased 15 minutes after injection (21, 25).

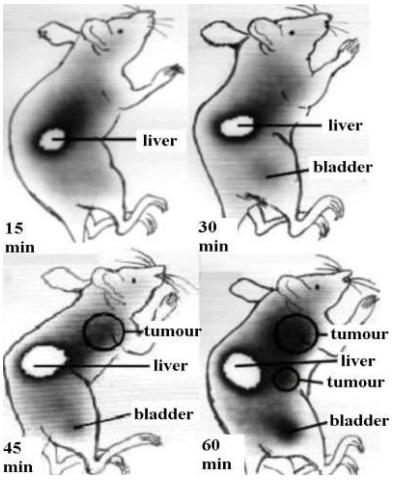

Fig. 4. Sequential scintigraphy at 15 minutes reveals the biodistribution of the marked compound (radiopharmaceutical)

In conclusion, the relatively weak bond between the pteridine nucleus and

the p-aminobenzoyl glutamic acid of MTX makes it possible to obtain a 2.4 diamino pterinic compound labeled with the 99 mTc radioisotope. The labeling process can take place by replacing p-aminobenzoyl glutamic acid with sodium pertechnetate in the oxidation state (VI).

A simple and effective method of highlighting substitution is RC using gel filtration. The use of this method of analysis allows the exact collection of fractions to determine the maximum radioactivity fraction corresponding to the labeled compound for further identification. The determination of the chemical nature of the radiopharmaceutical is thus done with the help of absorption spectroscopy by comparing the obtained spectra with those of the pterin ring. Thus, the labeled compound obtained is most likely a 2,4-diamino 6 pertechnetate pterin. Its use in scintigraphic biodistribution studies as a radiotracer in the form of a sterile pyrogen-free solution allows the determination of MTX pharmacokinetics "in vivo" by analogy in circumstances where animals cannot or must not be slaughtered.

Conclusions

The maximum intratumoral retention of pteridine compounds (including methotrexate) is reached within approximately one hour of i.v. Under these conditions, the use of Methotrexate as a photosensitizer is performed with maximum efficiency if irradiation of treated tumors (365 nm) is performed at least 60 minutes after administration of the cytotoxic agent. In addition, the radioactive substance obtained appears to have a very good antineoplastic therapeutic index, even in the absence of photostimulation.

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RESEARCH ON THE ECG WAVES' AMPLITUDE RECORDED IN GOAT USING LIMB LEADS

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Summary

The main aim of our research was to determine the amplitude of the ECG waves in goats. For this purpose, we investigated the limb lead system. After examining the obtained electrocardiograms, we found that the highest P wave amplitude is recorded in lead II (0.096 ± 0.045 mV) and lead I (0.089 ± 0.068 mV), the highest amplitude of the ventricular complex is recorded in lead III (0.253 ± 0.094 mV), lead II (0.242 ± 0.087 mV) and lead I (0.228 ± 0.110 mV), and the highest amplitude of the T wave is recorded in lead II (0.132 ± 0.060 mV) and lead I (0.128 ± 0.057 mV). The lowest electrocardiographic wave amplitudes were recorded in lead aVF with values of: 0.035 ± 0.049 mV for the P wave, 0.164 ± 0.045 mV for the QRS complex and 0.060 ± 0.044 mV for the T wave. For the ECG recording in goat using limb leads, we recommend the use of lead II, as it gives the highest amplitude, the recording obtained being easy to interpret.

Keywords: electrocardiography, goat, limb leads.

The electrocardiogram represents the recording of the cardiac electrical activity during the cardiac revolutions (3, 19). It is a non-invasive technique used in order to investigate the activity of the heart, providing information on the integrity of the excito-conducting system, the duration and frequency of the atrial and ventricular systole and assessment of the position of the heart (7, 13, 17). Also, the electrocardiogram can be used to assess the dimensions of the cardiac compartments and rhythm disturbances (2, 8, 18). The correct assessment of the electrocardiogram is of great practical importance, as it is recommended in clinics to calculate heart rate and MEA - mean electrical axis (9, 14, 15, 20). Compared to the situation in human medicine, where the methodology of performing and interpreting the electrocardiogram is standardized, in veterinary medicine there are several possibilities of recording the electrocardiogram because domestic animals have anatomical particularities, especially of the thoracic cavity, which is why precordial leads are practically unusable.

Studying the specialty literature, we found few papers concerning the amplitude of electrocardiographic waves recorded in goats, most authors being from Asia - where interest in breeding this species is also higher, studies being done on adult goats of different, usually local breeds (1, 5, 10, 11, 16). The main concern of

this study was to determine the amplitude of the electrocardiographic waves recorded using limb leads, following their appearance and recorded values (mV) for each lead. The values obtained were compared to see which lead allows an optimal recording. Our values were also compared with values reported by other authors.

Materials and methods

The necessary materials used in this research were electrocardiographs, metal traps and electrically conductive media. The electrocardiograph used - Innomed heart mirror - is a portable model, battery powered and allowed recording for each lead individually, that was chosen by pressing the specific buttons on the display. The paper used was BTL paper with a width of 6 cm. In the research we used alligator type captors because of their advantages, being easy to grip on the animal's skin, even if the hair is not trimmed. Regarding the electrically conductive media we used sanitary alcohol because it is easy to apply, it is not irritating to the skin, and the skin does not need to be cleaned after application, unlike gel which needs to be removed after examination.

The biological material used in our research consisted of 14 goats of the Carpathian breed, aged between 2 and 4 years, in the first part of the lactation period. In our research we recorded electrocardiograms using limb leads.

In order to record electrocardiograms in goats using limb leads, electrodes are placed on the body surface as follows: red electrode in the axillary area on the right side, yellow electrode in the axillary area on the left side, black electrode at in the groin area on the right side and green electrode in the groin area on the left side.

Note that the recordings were made at a speed of 25 mm/sec and the millivolt amplitude was 10 mm.

Results and discussions

Using limb leads we can record electrocardiograms in 3 bipolar leads (denoted I, II and III) and in 3 augmented unipolar leads (denoted aVR, aVL and aVF).

In Figures 1, 2, 3, 4, 5 and 6 we present the layout of the electrocardiograms recorded for each lead.

The analysis of the electrocardiograms recorded using limb leads shows that they are often contaminated with artefacts produced by the contraction of the limb muscles. Also, a low amplitude of the electrocardiographic waves is observed due to the large distance of the electrodes from the heart.

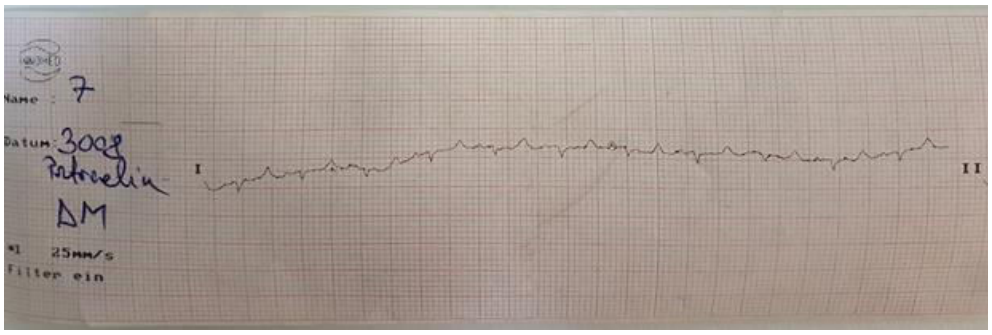

Fig. 1. ECG recorded in lead I

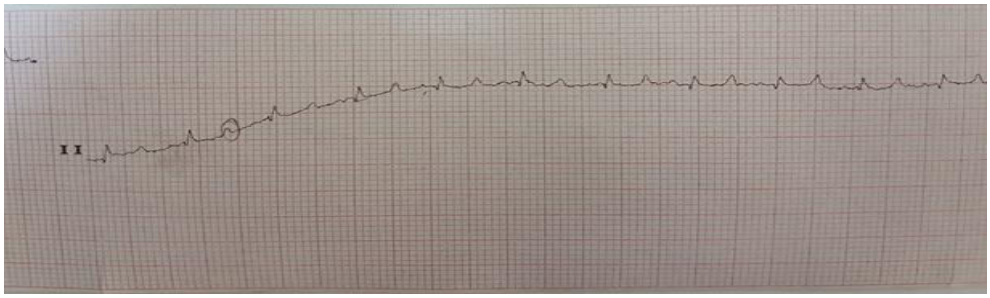

Fig. 2. ECG recorded in lead II

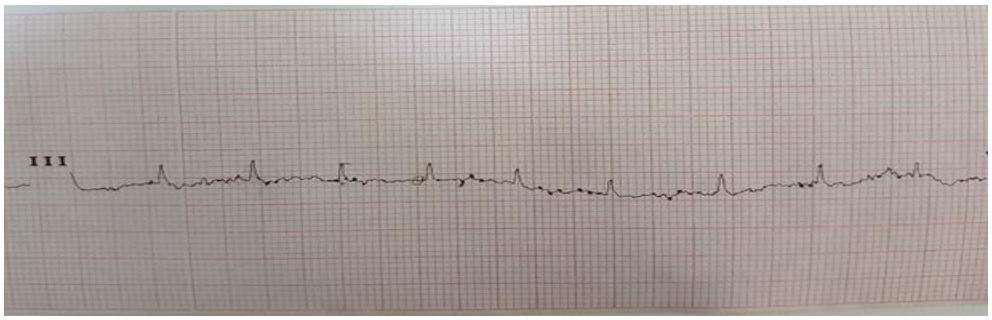

Fig. 3. ECG recorded in lead III

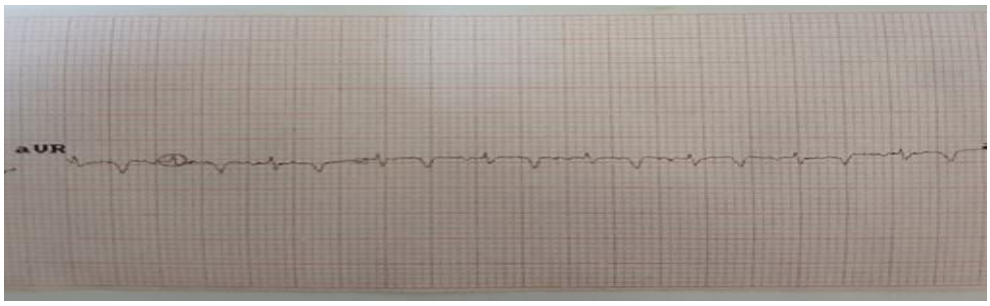

Fig. 4. ECG recorded in lead aVR

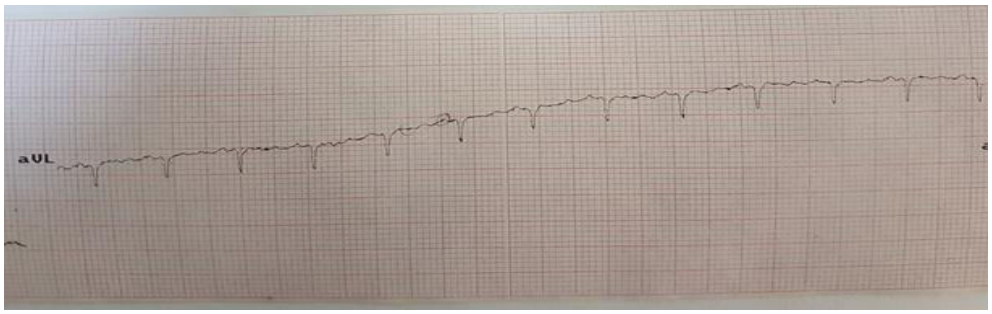

Fig. 5. ECG recorded in lead aVL

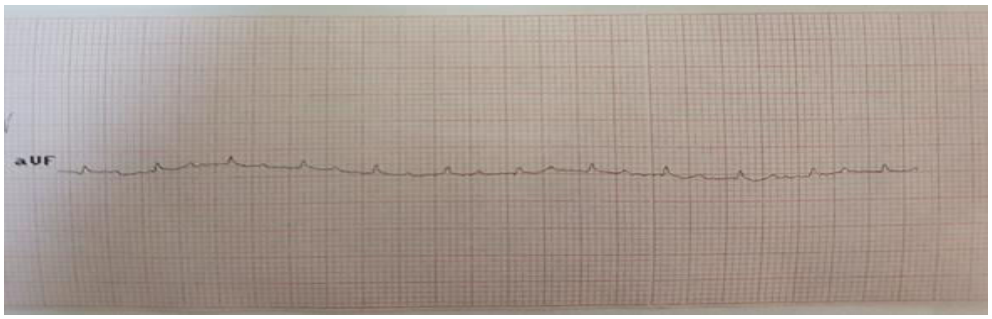

Fig. 6. ECG recorded in lead aVF

Regarding the amplitude of the electrocardiographic waves our results are shown in Tables 1, 2 and 3 and Figures 7, 8 and 9.

Table 1

P-wave amplitude values (mV) recorded using limb leads

No.	I	II	III	aVR	aVL	aVF
1	0.1	0.1	0.1	0	0	0.1
2	0.2	0.1	0	0.1	0	0
3	0.1	0.1	0.1	0.1	0.1	0
4	0	0	0	0	0.1	0.1
5	0.1	0.15	0	0.1	0	0
6	0.1	0.1	0.1	0.1	0	0
7	0	0.1	0	0	0.1	0
8	0.1	0.1	0.1	0	0	0.1
9	0.2	0.15	0	0.1	0	0
10	0.1	0.1	0.1	0.1	0.1	0
11	0	0	0.1	0	0.1	0
12	0.15	0.15	0	0.1	0	0.1
13	0.1	0.1	0.1	0.1	0	0
14	0	0.1	0	0	0.1	0.1
MEAN	0.089	0.096	0.051	0.057	0.042	0.035
Standard error	0.018	0.012	0.013	0.013	0.013	0.013
Standard deviation	0.068	0.045	0.051	0.051	0.051	0.049
Variance	0.004	0.002	0.002	0.002	0.002	0.002

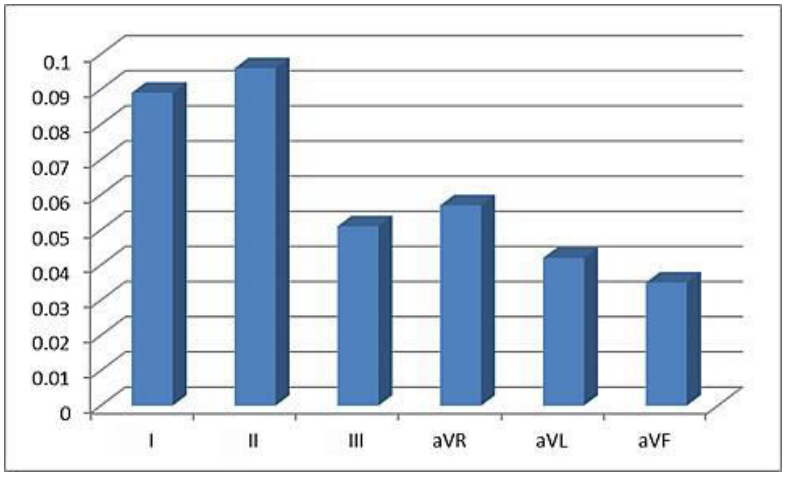

Fig. 7. Mean P-wave amplitude recorded using limb leads

From Table 1 and Figure 7 we can conclude that a very low amplitude of the P-wave is observed in all 6 leads, the average being less than 0.1 mV in all cases.

The highest amplitude is observed in lead II, with a mean value of 0.096 mV, the variability being small (variance 0.002 and standard deviation 0.045). Lead I has

a mean value of 0.089 mV, the variability being small (variance 0.004 and standard deviation 0.068). For all the other 4 leads, the amplitude is extremely small, the electrocardiogram being difficult to interpret.

Table 2

Ventricular complex amplitude values (mV) recorded using limb leads

No.	I	II	III	aVR	aVL	aVF
1	0.25	0.1	0.3	0.2	0.2	0.15
2	0.2	0.2	0.4	0.25	0.35	0.15
3	0.2	0.4	0.15	0.15	0.1	0.15
4	0.2	0.2	0.3	0.15	0.3	0.2
5	0.5	0.3	0.35	0.4	0.4	0.1
6	0.25	0.25	0.2	0.2	0.1	0.2
7	0.1	0.25	0.15	0.1	0.1	0.2
8	0.25	0.15	0.3	0.2	0.2	0.15
9	0.4	0.4	0.35	0.25	0.35	0.1
10	0.2	0.15	0.1	0.15	0.1	0.15
11	0.25	0.2	0.35	0.2	0.3	0.2
12 13	0.1 0.2	0.3 0.25	0.25 0.2	0.4 0.2	0.2 0.1	0.1 0.25
14	0.1	0.25	0.15	0.1	0.1	0.2
MEAN	0.228	0.242	0.253	0.211	0.207	0.164
Standard error	0.029	0.023	0.025	0.024	0.030	0.012
Standard deviation	0.110	0.087	0.094	0.092	0.112	0.045
Variance	0.012	0.007	0.009	0.008	0.012	0.002

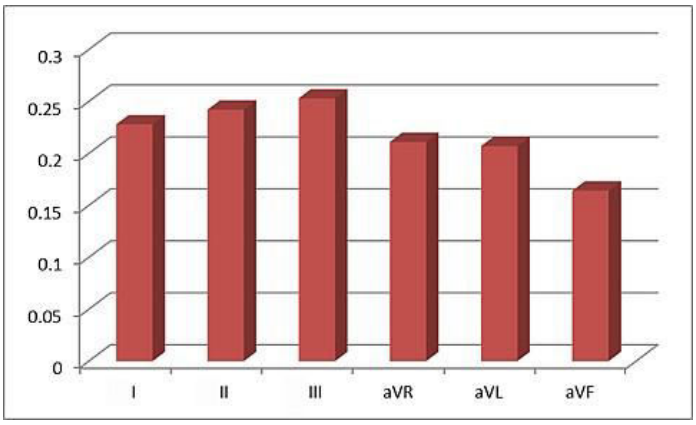

Fig. 8. Mean amplitude of the QRS complex recorded using limb leads

In Table 3 and Figure 9 we can observe the T-wave amplitude, the highest value is recorded in lead II, which is 0.132 mV, with low variability (0.003 variance and 0.060 standard deviation).

Table 3

T-wave amplitude values (mV) recorded using limb leads

No.	I	II	III	aVR	aVL	aVF
1	0.05	0.1	0.05	0	0	0.05
2	0.15	0.15	0.1	0.15	0.05	0.1
3	0.05	0.05	0	0.05	0	0
4	0.2	0.15	0.05	0	0.05	0.05
5	0.15	0.2	0	0.2	0.1	0.1
6	0.15	0.15	0	0.15	0.05	0.05
7	0.15	0.15	0.1	0.05	0.1	0.15
8	0.05	0.05	0.05	0	0.1	0.05
9	0.1	0.15	0.15	0.15	0.05	0.1
10	0.05	0.05	0	0.05	0	0
11	0.2	0.2	0.05	0	0.05	0.05
12	0.15	0.25	0	0.2	0.1	0.1
13	0.2	0.1	0.05	0.1	0.1	0.05
14	0.15	0.1	0.05	0	0.1	0
MEAN	0.128	0.132	0.046	0.078	0.063	0.060
Standard error	0.015	0.016	0.012	0.020	0.011	0.011
Standard deviation	0.057	0.060	0.045	0.077	0.041	0.044
Variance	0.003	0.003	0.002	0.006	0.001	0.001

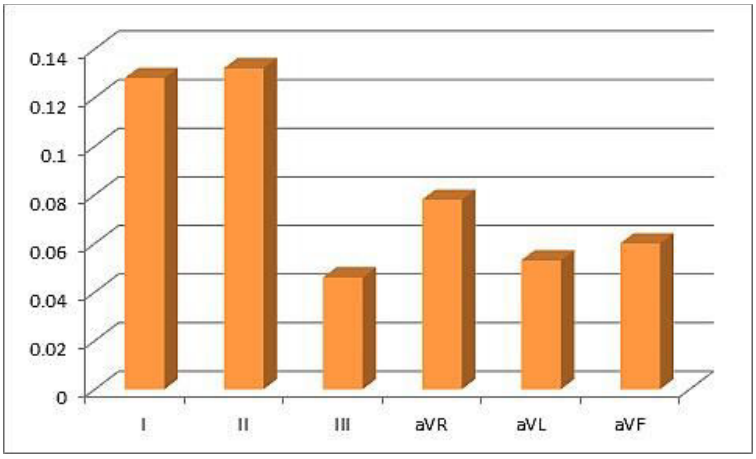

Fig. 9. Mean T-wave amplitude recorded using limb leads

Higher amplitudes are also observed in lead I, with a mean value of 0.128 mV (0.003 variance and 0.057 standard deviation). In all other 4 leads the T-wave amplitude is very low with mean values below 0.1 mV.

In Table 4 and Figure 10 we present the mean values of the amplitudes of all electrocardiographic waves, obtained in this study using limb leads.

Table 4

Mean electrocardiographic wave amplitudes (mV) recorded in the goat using limb leads

Wave	I	II	III	aVR	aVL	aVF
P	0.089	0.096	0.051	0.057	0.042	0.035
QRS	0.228	0.242	0.253	0.211	0.207	0.164
T	0.128	0.132	0.046	0.078	0.053	0.060

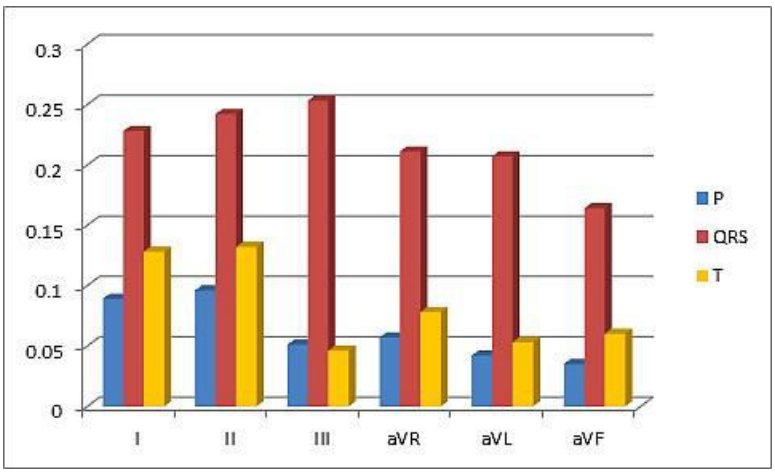

Fig. 10. Mean amplitudes of the P wave, QRS complex and T wave, recorded using limb leads

The analysis of the electrocardiograms recorded using limb leads shows that they provide an electrocardiographic trace that is frequently contaminated by artefacts produced by limbs' muscles contractions. Also, the amplitude of the electrocardiographic waves is quite small, which is why in many cases the electrocardiographic waves are difficult to detect on the recording. Of the limb leads that can be used to record electrocardiograms in goats, lead II (which gives the highest amplitude of the P and T waves), lead III (which gives the highest amplitude of the QRS complex) and lead I (which gives recordings with amplitudes that allow interpretation) can be used. The other leads provide electrocardiographic tracings with low amplitudes, making the electrocardiogram difficult to interpret.

Comparing our results with the results reported by other authors, we observe that they obtained the highest P-wave amplitude in lead I and aVR (5), in lead I (10) and in lead II and aVF (11), the highest QRS complex amplitude in lead I (6, 12), in lead III and aVR (1), in lead II and lead III (5) and in lead II (4), and the highest T-wave amplitude, in lead II (12), in lead II and lead III (1) and in lead III (11).

Conclusions

The limb leads that can be successfully used for ECG recording in goat are lead II and lead III, as they provide the highest amplitude to the electrocardiographic waves, ECG being easy to analyze and interpret.

Lead I, lead aVR and lead aVL have a limited recommendation for ECG recording in goats.

Lead aVF cannot be used for ECG recording in goats because it gives the lowest amplitude to the ECG waves, which is why the ECG cannot be interpreted.

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THERAPEUTIC MANAGEMENT OF A DOG WITH DIABETES MELLITUS AND ACUTE PANCREATITIS

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Summary

This case report presents the clinical evolution and therapeutic management of a dog which was diagnosed with diabetes mellitus and acute pancreatitis. A 9-year old male mixed breed dog was presented with weight loss, anorexia, vomiting and polyuria, polydipsia. During his life he was fed with commercial food (wet and dry) and home-cooked food. Physical examination revealed a cranial abdominal pain, body temperature of 39.4º C, respiratory rate 28 rpm, heart rate 127 bpm, medium dehydration and the mucous membranes having a normal color. Biochemical and hematological analyzes were performed. As a result, the following parameters showed significant increases: glucose, urea, creatinine, aspartate aminotransferase (AST), alanine transaminases (ALT), alkaline phosphatase, amylase and triglycerides. The test for canine pancreatic lipase (IDEXX Snap cPL*) was also positive. Leukocytosis, neutrophilia, and monocytosis were among the abnormalities found on the complete blood count. Urinalysis showed a normal specific gravity (1.032), glucosuria (1000 mg/dl), ketonuria (40 mg/dl), mild proteinuria (30 mg/dl). Abdominal ultrasound showed a hypoechoic pancreas area surrounded by hyperechoic peripancreatic fat, without other changes in the echogenicity of the organs in the abdominal cavity. The treatment for diabetes and acute pancreatitis was initiated and the clinical evolution was good, the dog being discharged 2 weeks after the beginning of therapy.

Keyword: pancreatitis, diabetes mellitus, dog.

Diabetes mellitus and pancreatitis are two separate disorders that are frequently seen in small animal medicine (4). While the clinical symptoms of diabetes mellitus are usually unmistakable (2), a definitive diagnosis of pancreatitis might be difficult to come by due to the variability of clinical indicators (7).

Pancreatitis is the most frequent exocrine pancreatic condition in dogs (3). It is difficult to diagnose clinically since the condition is usually moderate or subclinical (19), and its clinical symptoms are frequently the same as those of complicating or concomitant diseases (18).

Pancreatitis is frequently misdiagnosed until illness has progressed to the point that endocrine and/or exocrine insufficiency has developed, leading to diabetes mellitus (1). Comorbidities are common in dogs with diabetes, and some of them may have contributed to the diabetic state's development (20). Pancreatitis and diabetes mellitus (DM) have been reported to occur concurrently in many species – ranging from humans (17) to dogs and cats (12, 15), to a cow (9), and a sea lion (14).

Acute pancreatitis in dogs produces abdominal pain and also vomiting, and it can lead to life-threatening electrolyte imbalances, dehydration and disseminated intravascular coagulation. It has been reported that pancreatitis in dogs may generate β-cell damage, with the subsequent release of antigens triggering an immunological response that may accelerate islet destruction (8).

Generally, a definitive diagnosis of pancreatitis is established based on history, physical examination findings (13), and a combination of clinical pathologic (6) and imaging findings (11).

In this case report is presented the clinical evolution and therapeutic management of a dog diagnosed with diabetes mellitus and acute pancreatitis.

Materials and methods

The study was performed on a 9-year old male mixed breed dog presented with anorexia, weight loss, polyuria, polydipsia and vomiting, at the Internal Medicine Clinic of the Faculty of Veterinary Medicine in Timisoara. After the physical examination, blood samples were collected for biochemical and hematological analyses. The complete blood count was determined using an automated hematology analyzers (Procyte Dx, IDEXX). Blood biochemical parameters, aspartate aminotransferase (AST), alanine transaminases (ALT), alkaline phosphatase, glucose, urea, creatinine, amylase and triglycerides, determined by usual methods, with automatic biochemistry analyzers (RX-daytona, RANDOX). Urinalysis was performed with urine strips (Reactif 11M, Nal von minden). Canine-specific pancreatic lipase activity was also assessed using the SNAP cPL test (IDEXX Laboratories, Inc). Abdominal ultrasound was performed with My lab 70 Vet XVG, E-Saote. Blood and urine samples were taken in the first day, at 24 hours and 72 hours later, to monitor the patient's progress.

Results and discussions

Physical examination revealed a cranial abdominal pain, body temperature of 39.4º C, respiratory rate 28 rpm, heart rate 127 bpm, medium dehydration and the mucous membranes having a normal color. The complete blood count showed leukocytosis (42.58 K/µL, reference interval 5.05-16.76 K/µL), neutrophilia (37.51 K/µL, reference interval 2.95-11.64 K/µL) and monocytosis (3.67 K/µL, reference interval 0.16-1.12 K/µL), thrombocytosis (534 K/µL, reference interval 148-484 K/µL).

Analysis of blood biochemical parameters revealed hyperglycemia, increased serum amylase, AST, ALT, ALP activity and increased serum creatinine, urea, phosphorus and triglyceride concentrations (Table 1).

Table 1

Results of blood biochemical parameters

Analysis	UM	Result	Reference interval
Total protein	g%	**6.91**	**5.4 - 7.6**
Albumin	g%	**3.13**	**2.5- 4.4**
TGO (AST)	U/l	**71.79**	**10 – 40**
TGP (ALT)	U/l	**180.33**	**10 - 60**
ALP	U/l	**3757.11**	**< 150**
Creatinine	mg%	**3.41**	**0.4 – 1.4**
Glucose	mg%	**988.21**	**60–117**
Urea	mg%	**274.20**	**10 - 25**
Amylase	U/l	**9320**	**< 1650**
Triglyceride	mg%	**667.29**	**100 - 300**
Phosphorus	mg%	**9.65**	**2.1 - 5**

Also, the canine specific pancreatic lipase SNAP cPL test result was positive. Urinalysis showed a normal specific gravity (1.032), glucosuria (1000 mg/dl), ketonuria (40 mg/dl), mild proteinuria (30 mg/dl). Abdominal ultrasonography revealed an enlarged hypoechoic pancreas area surrounded by hyperechoic peripancreatic fat, without other changes in the echogenicity of the organs in the abdominal cavity.

Correlating anamnestic information with clinical signs, changes in laboratory findings and abnormalities on ultrasound examination, the dog was diagnosed with acute pancreatitis and diabetes mellitus.

The dog was hospitalized and treatment was initiated with IV fluid therapies initiated with NaCl 0.9% solution and adjusted on the basis of regular monitoring of hydration status. Fluids are important to correct hypovolemia, dehydration, and electrolyte and acid–base imbalances as early as possible to prevent any systemic complications, which can be associated with a negative outcome. (10)

Crystalline insulin (Humulin R) was administered on the first day of treatment, with a dose of 0.1 U/kg i.m at an interval of 1 hour over 5 hours, after which, crystalline insulin was administered subcutaneously at a dose of 0.4 U/kg every 6 hours. Additionally, vomiting was also treated with maropitant (1 mg/kg, i.v.), being recommended as a first choice antiemetic due to its high efficacy and visceral analgesic properties (10). Abdominal pain was controlled with buprenophine (0.01 mg/kg).

Blood glucose was measured during the first 24 hours, every 2 hours, registering a progressive decline of glycemia from the initial value of 988 mg/dL to 235 mg/dL, after 24 hours (Fig. 1).

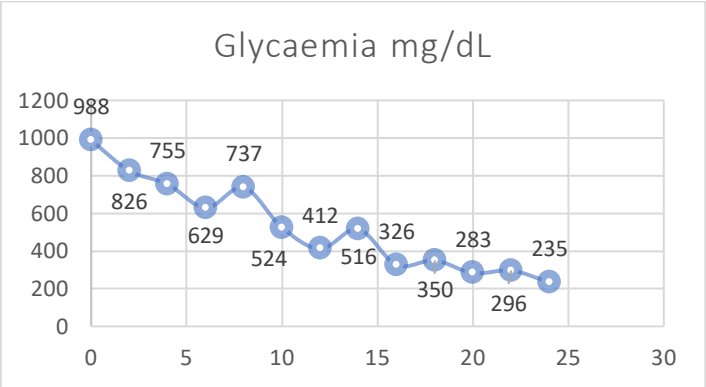

Fig. 1. Blood glucose evolution in the first 24 hours of insulin treatment

From the next day, Lantus insulin (Sanofi aventis) was administered at a dose of 0.5 IU/kg, once every 12 hours and blood glucose was measured before each administration. At 72 hours after the start of treatment, the blood glucose has registered values between 190 mg/dL and 286 mg/dL (Fig. 2).

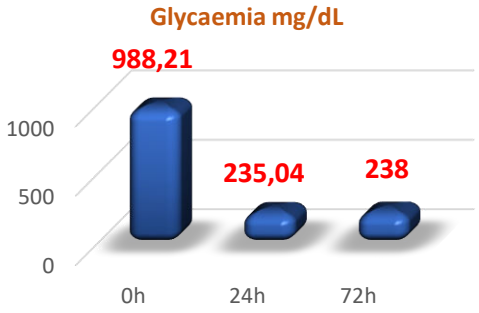

Fig 2. Serum glucose concentration

The initial value of serum amylase activity was 9320 U/L, after starting the treatment, at 24 hours its value decreased almost three times, reaching 3212 U/L and at 72 hours the amylase value reached within normal limits 1643 U / L (Fig. 3). In the presence of pancreatic inflammation, blood biochemical indicators such as amylase and lipase are usually, but not always, elevated. Serum amylase levels can be raised in dogs with diabetes, though this is not a consistent finding (3). For this reason was performed the canine specific pancreatic lipase SNAP cPL test which

showed a sensitivity of 91.5–94.1% and a specificity of 71.1–77.5%, and was considered as the most rapid diagnostic test for acute pancreatitis in dogs (11).

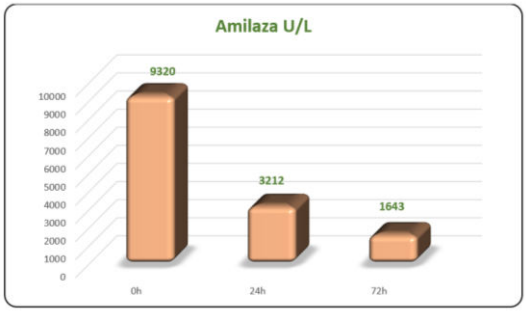

Fig. 3. Serum amylase values

The initial value of ALT was 180.33 U/L, at 24 hours its value decreased to 117.11 U/L while at 72 hours it increased, reaching 168.02 U/L. The initial value of AST was 259.02 U/l, at 24 hours it decreased to 158.65 U/L, at 72 hours recording an even more significant decrease, having the value of 71.79 U/L (Fig. 4).

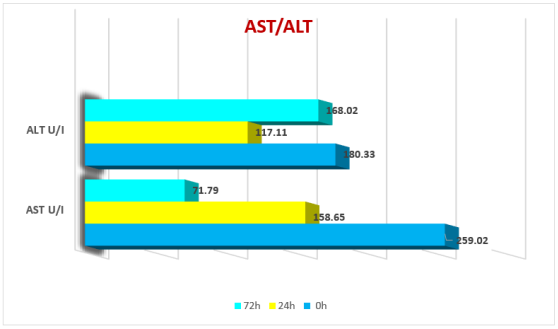

Fig. 4. Serum ALT and AST values

As regards creatinine, urea and triglycerides the initial value and those obtained at 24 and 72 hours are shown in the table below (Table 2). Even if the starting values were elevated at the beginning of the treatment, after 72 hours they returned almost within normal limits. This evolution suggests that the azotemia in this case had a pre-renal cause. Monitoring renal function in patients with acute pancreatitis is important because azotemia has been associated with increased risk

of mortality (12). The association between acute renal failure and acute pancreatitis has also been reported as a negative prognostic factor (16).

Table 2

Evolution of biochemical parameters

Parameter	**UM**	**Normal Value**	**0 h**	**24 h**	**72 h**
Creatinine	mg%	**0.4 – 1.4**	3.14	1.46	1.00
Urea	mg%	**10 - 25**	274.20	159.58	30.81
Triglyceride	mg%	**100-300**	667.29	81.85	110.25

The total leukocyte count decreased during the first 3 days of treatment from 42.58 K/µL to 25.56 K/µL after 72 hours. A decreasing trend was also observed for the absolute number of neutrophils, monocytes and thrombocytes (Table 3).

Table 3

Evolution of complete blood count parameters

Parameter	**Reference interval**	**0h**	**24h**	**72h**
WBC	5.05-16.76 K/µL	42.58	24.85	25.56
Neutrophil	2.95-11.64 K/µL	37.51	17.85	21.85
Lymphocyte	1.05-5.10 K/µL	1.07	1.26	1.80
Monocyte	0.16-1.12 K/µL	3.64	5.68	1.32
Eosinophil	0.06-1.23 K/µL	0.27	0.05	0.53
Basophil	0.00-0.10 K/µL	0.09	0.01	0.06
Platelets	148-4840 K/µL	534	405	365

Urinalysis revealed a normal urine specific gravity (1.030), glucosuria and ketonuria. Glucosuria, is highly suggestive of diabetes mellitus, occur after blood glucose exceeds the renal elimination level, which is approximately 180-220 mg/dl in dogs (5). Urine culture was also negative. The initial value of glucose in the urine was 1000 mg/dl (3+), after 24 hours of treatment was 500 mg/dl (2+) decreasing up to 250 mg/dl (1+) after 72 hours. The concentration of the ketone bodies in the urine was 40 mg/dl at initial evaluation, after 24 hours, dropped to 5 mg/dL and after 72 hours were not detected (Fig. 4).

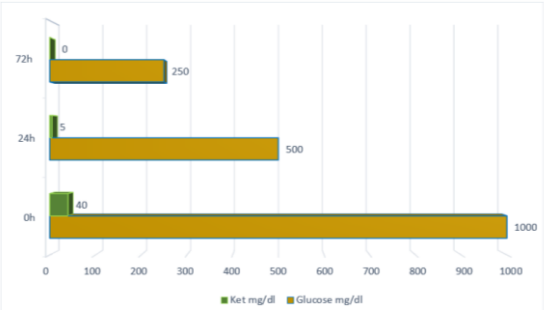

Fig. 4. Glucosuria and ketonuria dynamics in the first 72 hours

An extra low fat diet was prescribed in order to prevent a recurrence of acute pancreatitis, and any treats with a high fat content were discouraged. The dog was discharged after 10 days of treatment, in a stable condition, with the recommendation that Lantus insulin be administered at a dose of 0.6 U/kg every 12 hours.

Both diabetes mellitus and pancreatitis are complex diseases. As the endocrine and exocrine tissues of the pancreas are so interlinked, it is not surprising that damage to one or the other has an impact on the surrounding tissues.

Conclusions

Frequent monitoring of blood and urine biochemical parameters helps to modulate treatment according to clinical course and may improve the chances of survival of dogs with diabetes and acute pancreatitis.

Canine acute pancreatitis can be a reversible disease when diagnosed promptly and managed appropriately.

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STUDY REGARDING A COMPUTER APPLICATION DESIGNED TO MANAGE A CATTLE BREEDING PROGRAM IN ROMANIA

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Abstract

In Romania, as a result of favorable factors, such as natural pasture potential, european subsidies, government programs for the purchasing animals, estensive intensive growth technology systems, the price of bovine meat, population trends towards the consumption of meat obtained in ecological conditions, in Romania were imported a series of specialized breeds for meat production, including Aberdeen Angus, Galloway, Highland, Aubrac, Charolais, Limousin. Aberdeen Angus breed was imported in Romania at the end of 2008 in Sibiu county when about 120 heifers were imported from Germany. In our country, the development and monitoring of this breed is carried out by the Aberdeen Angus Association, from Sibiu, this being the only accredited association for the services of drawing up and maintaining the heerdbook of the Aberdeen Angus breed in Romania. Togheter with the implementation of european laws, according to the EU Regulation 1012 08 06 2016 O M 19 2016 and the Aberdeen Angus Breeding Program at the national level, the association undertook to develop a computer program capable of centralizing, storing and evaluating information of this breed on Romania. The BIDAA computer application (Informatic Database of Aberdeen Angus)) was developed at the initiative of the Aberdeen Angus Association from Sibiu, the leader of herdbookhead for the Aberdeen Angus breed inRomania. Gennerally in this paperwork it wiil be studied how the BIDAA application worksfor all target groups and how useful the it is for this specific field of activity.

Keywords: Aberdeen Angus, beef cattle, computer application, BIDAA, herdbook.

Mankind is currently going through a period in which all things are moving faster and faster, we want as much and of course and as qualitatively as possible. The agricultural area, and in this case the zootechnical one, is not bypassed by this trend and in view of the fact that the food sector according to Maslow's pyramid is in the first stage of human requirements (2, 3, 7). Thus, in order to keep up with the continuous evolution, animal husbandry sector has also focused on obtaining better results both qualitatively and quantitatively towards the main tools applicablefor this purpose, namely technology and digitization (1, 8, 10).

The raising of specialized cattle for meat production in Romania was until recently a total enigma for the livestock sector, but in recent years this activity has seen a continuous increase due to favorable factors such as: European and national subsidies, government financial programs for the purchase of animals purebred, declining human resources, unfair competition in the milk production department and, implicitly, the low price of milk, the existence at national level of significant areas of undervalued pasture (4, 18).

The Aberdeen Angus breed entered shyly but confidently according to the latest statistics on the territory of our country, the first contact of the breed being only in 2008 when the first flocks were brought to a farmin Sibiu County (11, 19). The breed register was officially taken over only in 2015 bythe Romanian Aberdeen Angus Association, which had to store all the influx of information about the ancestry of imported animals and then the performance ofthe offspring born in our country in an official national database (12, 13).

For this purpose, during 2018, the group of affiliated farmers of the Romanian Aberdeen Angus Association and the executive team of the association launched the BIDAA (Informatic Database of Aberdeen Angus), a computer program with the main purpose of storing all information about animals, their ancestors, performances (6).

Materials and methods

In order to develop the basic structure of the program and subsequently its continuous improvement, it was used to collect information from the following sources:

a. OFFICIAL PERFORMANCES CONTROL FOR MEAT PRODUCTION (O.P.C.). Romanian Aberdeen Angus Association is also accredited by the Ministry Of Agriculture for making official performances control for meat production on cattle, they perform these measurements at about 97% of the total herd, both purebred and under various crossbreeding programs in Romania (5, 6).Through the O.PC. technique, information regarding performances of the animals borned in Romania are gathered, respectively measurements of young bovine between 3 months and 14 months. The categories of animals weight are young calves, both males and females of both with the age between 90 and 410 days, and the interval between two consecutive weighings has a minimum value of 60 days and a maximum of 210 days (16, 20). Also throught O.P.C. information about breeding bulls are gathered, about the measurable aspects: lengths (length of the rump grip the tail), depth (chest depth), heights (height at the rump / croup), scrotal circumference, live weight.

Calculation method in suckler herds from birth to weaning

A.D.G. = (WW-BW)*1000/AW

A.D.G. – average daily gain WW - live weight at weaning

BW – birth weight AW – age of weaning

Calculation method in finishing herds after weaning to slaughter

A.D.G. = (Wn-1-Wn)*1000 / (An-1 -An) An-1 - age at weight recording n-1

An - age at weight recording n

Wn-1 - live weight at weight recording n-1Wn - live weight at weight recording n

b. PEDIGREES. Another essential source of information in consolidating the general database of the program was the certificates of origin of the imported biological material in the form of cows, calves, heifers, bulls as well as in the form of semen or embryos (15, 17).

ASOCIAŢIA ABERDEEN ANGUS ROMÂNIA

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REGISTRUL GENEALOGIC AL RASEI ABERDEEN ANGUS

CERTIFICAT ZOOTEHNIC

ICAR MEMBER

RASA (Breed): Aberdeen Angus

NUME (Name): AHF HOUSE BOY W948

NUMĂR MATRICOL (ID): RO50700995294 8

IDENTIFICARE: Crotalie auriculară

DATA NAŞTERII (Date of birth): 12.10.2020

RG (Herd book): A

Număr R.G. (Herd book number): 156032

SEX (Gender): Mascul

CULOARE (Color): Negru

ADN (DNA): 0202822

PP (Personal performance):

	Greutate/SMZ(weight/ADG)	EBV	PEBV	Acc
GN (birth weight)	39kg	3.39	108.68	66.69
G200 (weight 200)	319kg/1400g	35.27	110.80	68.13
G365 (weight 365)	563kg/1436g	93.07	122.53	65.58
EBV Total		42.03	110.78	46.89

CREŞCĂTOR (Breeder): ANGUS HOUSE FARM S.R.L., Ic̦lod, CLUJ

PROPRIETAR (Owner): ASOCIAŢIA ANGUS S.R.L., Fântânele, MUREŞ

TATA/*Sire*: HOUSE BOY T043
Număr matricol (ID): RO502007009043
Data naşterii (Date of birth): 10.04.2017 RG: A
Rasa (Breed): Aberdeen Angus
EBV:
PP: GN (birth weight):41;SMZ365(ADG):1094;

MAMA/*Dam*: WINGFIELD KATHIE PRIDE P565
Număr matricol (ID): UK260551500565
Data naşterii (Date of birth): 08.05.2014 RG: A
Rasa (Breed): Aberdeen Angus
EBV:
PP:

TT/*Paternal Grand Sire*: NETHERTON EUROPEAN
Număr matricol (ID): UK542697300788
Data naşterii (Date of birth): 14.12.2013 RG: A
EBV:
PP:

MT/*Paternal Grand Dam*: DORA
Număr matricol (ID): RO500002144778
Data naşterii (Date of birth): 13.07.2013 RG: A
EBV:
PP:

TM/*Maternal Grand Sire*: TONLEY ANELKA J617
Număr matricol (ID): UK520423700617
Data naşterii (Date of birth): - RG: A
EBV:
PP:

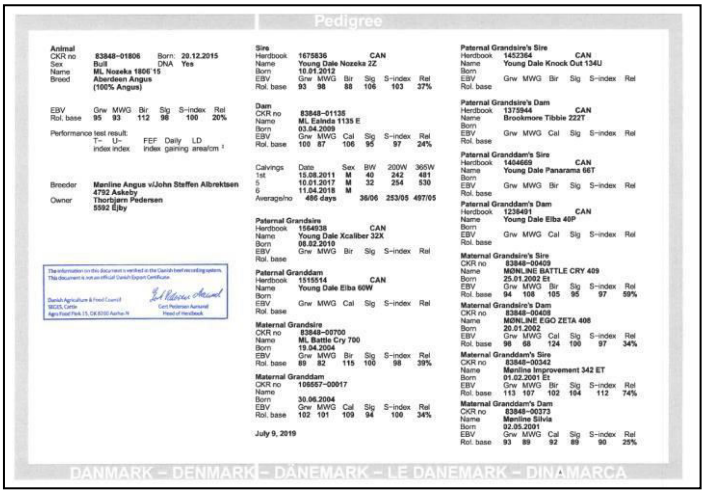
MM/*Maternal Grand Dam*: WINGFIELD KATHIE PRIDE
Număr matricol (ID): UK260551300353
Data naşterii (Date of birth): - RG: A
EBV:
PP:

Documentul numărul (Document number): 22663
Întocmit la data de (Drafted on): 04.04.2022

Operator: GOCIMAN IOAN TEODOR
Funcţie: Director tehnic executiv

Certificat zootehnic eliberat în conformitate cu Regulamentul nr. 1012 din 08 iunie 2016 al Parlamentului European şi al Consiliului Uniunii Europene, cu modificările ulterioare.
Zootehnic Certificate issued in accordance with the Regulation no. 1012 of June 8th 2016 of the European Parliament and of the European Union Council, as amended and supplemented.

Fig. 1. Romanian zootechnical certificate (pedigree)



Pedigree

Animal
CKR no 83848-01806 Born: 20.12.2015
Sex Bull DNA Yes
Name ML Nozeka 1806 15
Breed Aberdeen Angus (100% Angus)

EBV Grw MWG Bir Sig S-index Rel
Rol. base 95 93 112 98 100 20%

Performance test result: T= U= FEF Daily LD
index index index gaining area/cm ²

Breeder Manline Angus v/John Steffen Albrektsen
Owner 4792 Askeby Thorbjørn Pedersen 5592 Ejby

Sire
Herdbook 1675836 CAN
Name Young Dale Nozeka ZZ
Born 10.01.2012
EBV Grw MWG Bir Sig S-index Rel
Rol. base 93 98 88 106 103 37%

Dam
CKR no 83848-01135
Name ML Ealnda 1135 E
Born 03.04.2009
EBV Grw MWG Cal Sig S-index Rel
Rol. base 100 87 106 95 97 24%

Calvings Date Sex BW 200W 365W
1st 15.08.2011 M 40 242 481
5 10.01.2017 M 32 254 530
6 11.04.2018 M
Average/no 486 days 36/06 253/05 497/05

Paternal Grandsire
Herdbook 1564938 CAN
Name Young Dale Xcaliber 32X
Born 08.02.2010
EBV Grw MWG Bir Sig S-index Rel
Rol. base

Paternal Granddam
Herdbook 1515514 CAN
Name Young Dale Elba 60W
Born
EBV Grw MWG Cal Sig S-index Rel
Rol. base

Maternal Grandsire
CKR no 83848-00700
Name ML Battle Cry 700
Born 19.04.2004
EBV Grw MWG Bir Sig S-index Rel
Rol. base 89 82 115 100 98 39%

Maternal Granddam
CKR no 106557-00017
Name
Born 30.06.2004
EBV Grw MWG Cal Sig S-index Rel
Rol. base 102 101 109 94 100 34%

July 9, 2019

Paternal Grandsire's Sire
Herdbook 1452364 CAN
Name Young Dale Knock Out 134U
Born
EBV Grw MWG Bir Sig S-index Rel
Rol. base

Paternal Grandsire's Dam
Herdbook 1375944 CAN
Name Brookmore Tibbie 222T
Born
EBV Grw MWG Cal Sig S-index Rel
Rol. base

Paternal Granddam's Sire
Herdbook 1404669 CAN
Name Young Dale Panarama 66T
Born
EBV Grw MWG Bir Sig S-index Rel
Rol. base

Paternal Granddam's Dam
Herdbook 1236491 CAN
Name Young Dale Elba 40P
Born
EBV Grw MWG Cal Sig S-index Rel
Rol. base

Maternal Grandsire's Sire
CKR no 83848-00409
Name MØNLINE BATTLE CRY 409
Born 25.01.2002 Et
EBV Grw MWG Bir Sig S-index Rel
Rol. base 94 108 105 95 97 39%

Maternal Grandsire's Dam
CKR no 83848-00408
Name MØNLINE EGO ZETA 408
Born 20.01.2002
EBV Grw MWG Cal Sig S-index Rel
Rol. base 96 68 124 100 97 34%

Maternal Granddam's Sire
CKR no 83848-00342
Name Manline Improvement 342 ET
Born 01.02.2001 Et
EBV Grw MWG Bir Sig S-index Rel
Rol. base 113 107 102 104 112 74%

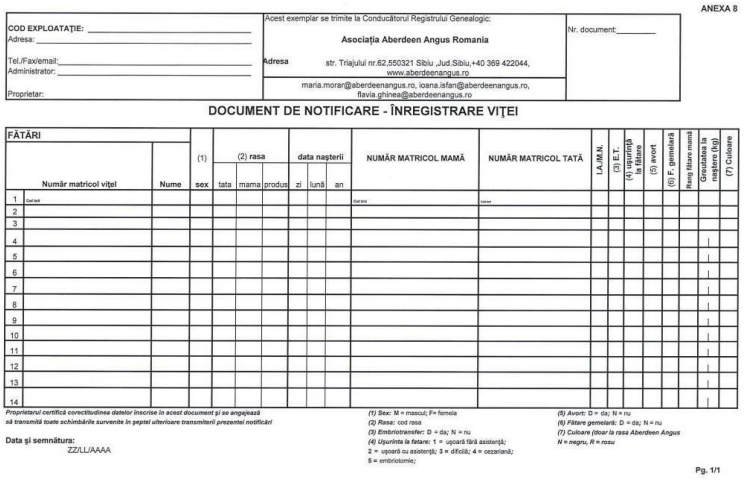
Maternal Granddam's Dam
CKR no 83848-00373
Name Manline Silvia
Born 02.05.2001
EBV Grw MWG Cal Sig S-index Rel
Rol. base 93 89 92 89 90 25%

DANMARK – DENMARK – DÄNEMARK – LE DANEMARK – DINAMARCA

Fig. 2. Denmark zootechnical certificate (pedigree)

c. DOCUMENTS OF THE FARMERS. The third source of information are farmers who are required to submit every three months to the office of the O.P.C.

department - the database of the association, the events produced on the farm according to specific notification documents: breeding/ artificial insemination registration (Annex 3), embryo transfer (Annex 4), calf registration (Annex 8), inputs/ outputs (Annex 10).



ANEXA 8

COD EXPLOATAŢIE: _____
Adresa: _____
Tel./Fax/email: _____
Administrator: _____
Proprietar:

Acest exemplar se trimite la Conducătorul Registrului Genealogic:
Asociaţia Aberdeen Angus Romania
Adresa: str. Trialului nr.62,550321 Sibiu ,Jud.Sibiu,+40 369 422044, www.aberdeenangus.ro
maria.morar@aberdeenangus.ro, ioana.iafan@aberdeenangus.ro, flavia.ghinea@aberdeenangus.ro

Nr. document: _____

DOCUMENT DE NOTIFICARE - ÎNREGISTRARE VIŢEI

FĂTĂRI																				
	Număr matricol viţel	Nume	(1) sex	(2) rasa tata	mama	produs	data naşterii zi	lună	an	NUMĂR MATRICOL MAMĂ	NUMĂR MATRICOL TATĂ	I.A./M.N.	(3) E.T.	(4) ispunită la fătare	(5) avort	(6) F. gemelară	Rasa fătare mama	Greutatea la naştere (kg)	(7) Culoare
1	Dati text									Dati text	Dati text								
2																			
3																			
4																			
5																			
6																			
7																			
8																			
9																			
10																			
11																			
12																			
13																			
14																			

Proprietarul certifică corectitudinea datelor înscrise în acest document şi se angajează să transmită toate schimbările survenite în peptel ulterioare transmiterii prezentei notificări

Data şi semnătura:
ZZ/LL/AAAA

(1) Sex: M = mascul; F= femela
(2) Rasa: cod rasa
(3) Embriotransfer: D = da; N = nu
(4) Ușurința la fatare: 1 = ușoară fără asistență; 2 = ușoară cu asistență; 3 = dificilă; 4 = cezariană; 5 = embriotomie;
(5) Avort: D = da; N = nu
(6) Fătare gemelară: D = da; N = nu
(7) Culoare (doar la rasa Aberdeen Angus N = negru, R = roșu

Pg. 1/1

Fig. 3. Calf registration (annex 8)

Results and discussions

In order to observe the evolution of the program, the reports related to: the evolution of farms (Fig. 4), the evolution of herds (Fig. 4), control activity (Fig. 5), genealogical register activity (Fig. 5), evolution of reproductive parameters (table 1), evolution of production parameters (Table 2), evolution of indices for estimating the improvement value were followed (Table 3).

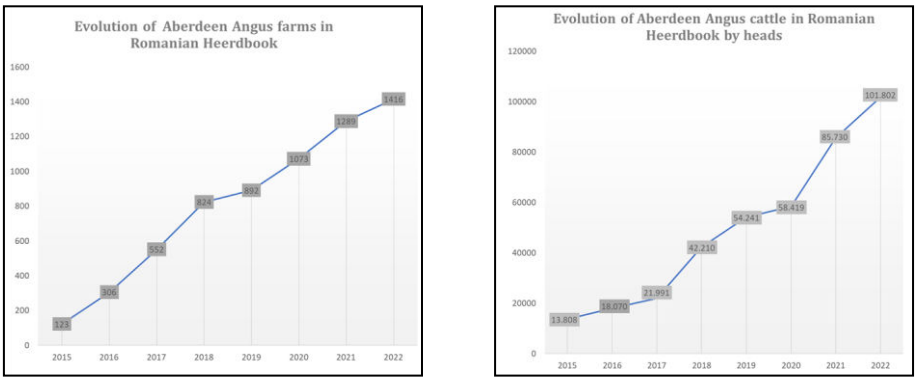


Fig. 4. Evolution of Aberdeen Angus farm and the stocks at national level

Regarding the evolution of Aberdeen Angus cattle stocks at national level, according to the data registered in the genealogical register department of the Romanian Aberdeen Angus Association, at the beginning of the year 2022 our country registered a total of 101,802 heads. Also according to statistical data provided by the Romanian Aberdeen Angus Association in 2015, they were registered with the OPC a total of 123 farms, 77% of which were under the control of the Romanian Aberdeen Angus Association. At the beginning of 2022, the same institution records a total of 1416 farms under control, of which a share of 3.4% of farms being verified by other associations.

Table 1

Aberdeen Angus stocks by physiological categories, in 2022

Specification	Total cows	Total cows PB	Total cows HB	Total heifers	Total heifers PB	Total heifers HB	Total breeding bulls	Total stock
Unit	heads	heads	heads	heads	heads	heads	heads	heads
TOTAL	39101	29553	9548	74091	51851	22240	1434	145368

* PB – purebreed/ * HB – half breed

Table 2

Evolution of average weight and daily gain per years

Specification	Unit	2018	2019	2020	2021
B.W.	kg	28	29	30	30
G7	kg	208	208	214	215
G10	kg	278	278	281	280
G12	kg	327	315	311	313
A.D.G. G7	g	892	887	917	929
A.D.G. G10	g	816	828	880	834
A.D.G. G12	g	791	780	770	775

* BW – birth weight/ ADG – average daily gain

Table 3

Estimating breeding values average recorded in 2022

Specification	V.A.A.	V.A.R.	ACCURACY
Unit	*kg*	*%*	*%*
Birth weight	+0.117	100.13	65.17
7 months weight	+1.048	99.97	64.95
10 months weight	+1.022	99.19	61.58
12 months weight	+2.283	99.35	62.64
Global Index	+0.61	99.55	30.81

Thus, from 2018 to 2021, a continuous increase in average weight and daily gain at the age of 7 months, from 208 to 215 kg, respectively from 892 to 929 grams / day. And in the case of the 10 months category, an increase in both weight and daily gain from 816 to 834 grams / day. The 12 months category is the one that has a continuous decrease from 791 to 775 grams / day, a fact which is influenced by certain technological factors such as: age at weaning, method of weaning, weight at weaning date, allotment, feeding before and after weaning.

An animal's breeding value is its genetic merit, half of which will be passed on to its progeny. While we will never know the exact breeding value, for performance traits it is possible to make good estimates. These estimates are called Estimated Breeding Values (EBVs). In the calculation of EBVs, the performance of individual animals within a contemporary group is directly compared to the average of other animals in that group. A contemporary group consists of animals of the same sex and age class within a herd, run under the same management conditions and treated equally. Indirect comparisons are made between animals reared in different contemporary groups, through the use ofpedigree links between the groups (9, 20).

Total selection index: EBV Total = 0% * EBV birth + 40% * EBVweaning-200 days + 30% * EBV 300 days+30% * EBV 365 days.

V.A.A. - the value of absolute improvement represents the positive or negative deviation of the population average at a certain character, thus the population average being generic 0 any positive deviation (ex: +0.5 kg) is a plus for the population, instead any negative deviation (-0.2 kg) is a minus in the population.

V.A.R. - the value of relative improvement represents the performance of an animal as a percentage difference from the population average, so any value above the generic 100% is a plus in the population and any value below is a minus in the population.

Accuracy - represents the percentage accuracy of this value indicator (6).

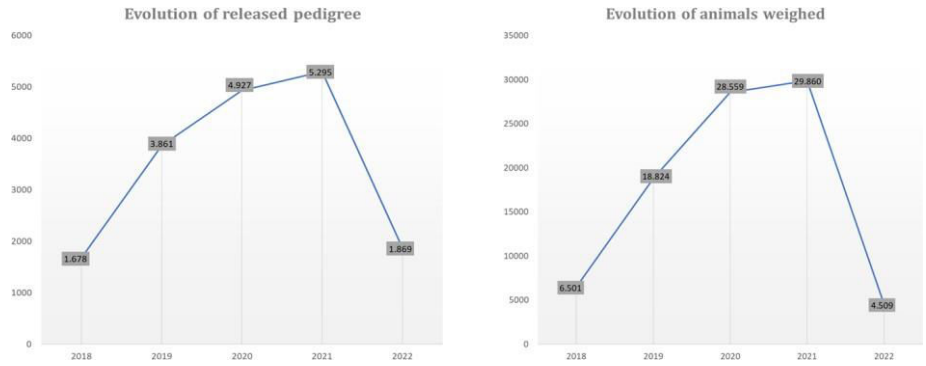


Fig. 5. Evolution of O.P.C. and heerdbook activity at national level

Conclusions

The results of the investigation show us the strengths of this sector respectively: the number heads at national had increase constantly which is favorable for future selection work and in terms of performances we also observe a continuous evolution which approaches to the standards set by the breeding program of Aberdeen Angus breed at national level. Thus in order to increase quantity and quality on this breed, cattle producers can benefit with this programe by a comprehensive range of information regarding the genetic merit of an animal.

Acknowledgement

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TESTING THE EFFECTIVENESS OF TWO THERAPEUTIC PROTOCOLS IN INFECTED LAMBS FROM VALCEA COUNTY

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Summary

Protozoa and cestodes are found in sheep farms and have an increased susceptibility to lambs. The clinical effects of this endoparasitosis on sheep, but especially on lambs, lead to weight loss and even mortality. When assessing situations of polyparasitism in lambs, the youngest age group, we must choose the right substances according to the specific epidemiological situation and the intrinsic and extrinsic factors that may influence their therapeutic efficacy. In this context, the aim of the present study was to evaluate the therapeutic efficacy of two protocols administered to lambs from a Valcea County farm, infested with protozoa and cestodes. We performed the treatment based on Toltrazuril, respectively Diclazuril, which proved to be effective against *Eimeria spp.*, the E.P.G.-s decreased significantly, with a higher efficacy attributed to the Baycox product (Toltrazuril). The descriptive statistics associated with the scores corresponding to the E.P.G. obtained in the coprological examination and the application of the Kruskal Wallis test to determine the effectiveness of the treatment support the recommendation of the combination of Praziquantel (Prazicest) and Toltrazuril (Baycox 5%) in lambs infested with Eimeria spp. and tapeworms.
Keywords: lambs, Praziquantel, Toltrazuril, Valcea County.

Gastrointestinal nematodes (GIN) are a major health problem for sheep. Young growing lambs are most susceptible to parasite infestation, but breeding sheep are also more susceptible to the negative effects of parasite infestation in the period immediately before and after calving (25).

Eimeriosis is a protozoosis that occurs on farms with poor growth and housing conditions and can cause significant losses due to high morbidity, mortality, and weight loss. Susceptibility is increased in lambs 2-6 months of age, where mortality or reduced body weight occurs. Clinical expression is seen in 6-12 week old lambs after grazing (8).

Cestodosis, caused by the *Anoplocephalidae* family cestods occurring in the intestine and less commonly in the bile ducts, usually occurs in young lambs and is associated with unattained weight loss. The disease occurs seasonally, from spring

to summer, and is related to the presence of intermediate hosts in the form of grass mites on pasture (20, 22, 26).

The clinical impact of this endoparasitosis in sheep, but especially in lambs, the choice of the most effective therapeutic protocol to control this infestation, but also to avoid the occurrence of chemotherapy, responsible management of grassland that does not favor the development of spores and intermediate hosts, increasing the efficiency of deworming to maintain the level of parasitic to an economically harmless degree is important desiderata of a parasitological control.

In the evaluation of polyparasitism situations in the youngest age group, the lambs, we must select the right substances according to the concrete epidemiological situation and the intrinsic and extrinsic factors that may influence their therapeutic efficacy.

In this context, the purpose of this study was to evaluate the therapeutic efficacy of two therapeutic protocols administered to lambs a farm in Valcea County, infested with protozoa and tapeworms.

Materials and methods

The study was conducted on a private farm with a population of 180 lambs of the Turcan breed 2-4 months aged. The animals are maintained on dry pastures, but also wet, with/ without swampy land (Fig.1).

Fig. 1. The area of the provenance of the samples

Between 50 and 150 g of freshly discarded faeces were collected or directly from the rectum of the goats under study. The samples were collected and refrigerated until processing. Gastrointestinal masses and organs (lung, liver, CNS) were also harvested.

The samples have been processed at the Parasitology and Parasitic Diseases Clinics of the Faculty of Veterinary Medicine Timișoara by the following methods:

- Qualitative method - identification of the parasitic load with light eggs of nematodes, cestodes, protozoan oocysts.
- Polyvalent method (of successive washes) - identification of the presence of trematode eggs.
- Larvoscopic method - highlighting parasitism with pulmonary nematodes.
- Necropsy examination - in slaughtered individuals, according to the technical instructions of necropsy (Fig. 2, Fig. 3) (11).

Fig. 2. Macroscopic examination of organs

Fig. 3. Necropsy examination

The intensity of infestation was assessed by the number of parasitic elements identified in the microscopic field:

- no infestation 0 elements/field ("-");
- weak infestation 1-2 elements/field ("+");
- medium infestation 3-5 elements/field ("+ +");
- heavy infestation 5-10 elements/field ("+ +");
- massive infestation, more than 10 elements/field ("+ + +").

The MC Master method was performed on days 0 and 14 after treatment, and the EPG was determined according to the calculation formula E.P.G.= nx100/2, where "n" represents the number of eggs found in both chambers of the McMaster blade (11).

The methods for calculating the efficacy of the anthelmintic used were performed according to the relationship of Presidente and Boorgsteede (11).

Presidente relationship (%): [1-T2/T1xC1/C2]x100; where T1 and T2=E.P.G.

lot treated day 0 (T1) and day 14 (T2) and C1 and C2=E.P.G. lot control day 0 (C1) and day 14 (C2).

Borgsteede ratio (%): (1-T2/T1xGlobal mean subject day 0/mean lot day 14 blank)x100; where T1 and T2 - E.P.G. treated lot day 0 (T1) and day 14 (T2).

The studied groups were:

- Lot I, which was administered Albendakel 10% și Vecoxan.
- Lot II, which was treated with Prazicest și Baycox 5%.
- The control group received no treatment.
- The results obtained were statistically analysed by applying the Kruskal-Wallis test to determine the differences between the three groups.

Results and discussion

The results of the coprological examination performed by the flotation method revealed the presence of the following parasitic elements: oocysts of protozoa and oviger proglots (cestodes oncosfers) (Fig. 4, Fig. 5).

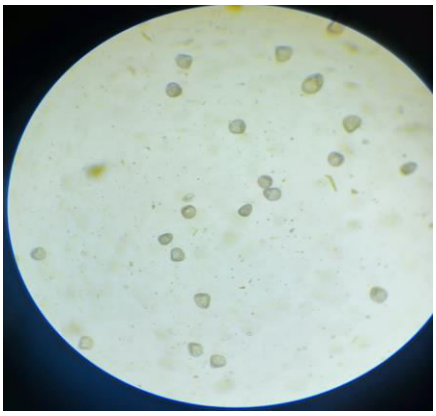

Fig. 4. Oviger proglots (cestodes oncosfers)

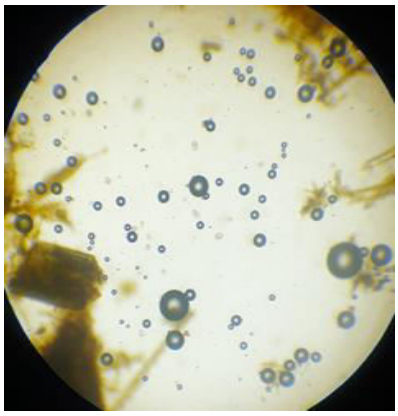

Fig. 5. Oocysts of protozoa

The results of the necropsy examination revealed the presence of *Moniezia expansa* (Fig. 6) in the small intestine of the lambs.

Fig. 6. *Moniezia expansa*

In group 1, the average value of E.P.G. on day 0 was 130 with cutoff values ranging from 50 to 300. On day 14, E.P.G. values in group 1 -animals treated with Albendakel 10% and Vecoxan, the E.P.G. values were 10. In group 2, the average E.P.G. was 150 with cutoff values ranging from 50 to 400. On day 14, the mean E.P.G. was 0 in group 2 - animals treated with Prazicest and Baycox 5%.

Box-plot plots were made with the values corresponding to the E.P.G. obtained, the median and standard deviation were calculated, and the Kruskal-Wallis test was applied to determine the efficacy of the treatment. Significant differences were found between the three groups ($p=0.000 < 0.05$ and $p=0.003$, respectively), with the highest therapeutic efficacy in favor of lot II, the group of lambs treated with Prazicest and Baycox 5%.

A widespread disease in all areas of the world where sheep are kept both on pasture and in semi-intensive housing is eimeriosis, a high-frequency protozoan infestation that affects lambs between 2-6 months of age and leads to mortality or reduced body weight. Adult sheep acquire resistance to infection and young dams can be a source of infection for lambs (2, 17).

Infestation occurs mainly in pasture, along with grass and water, with sporulated oocysts. Infestation of grass is unlikely and unimportant in shelters (21).

Oocysts are very resistant in the environment. They can persist in feces for several months. The shaded and cool areas around shelters, moist spaces around watering holes, the edge of streams provide good conditions for the spread of oocysts. Although some oocysts can survive the winter, they are not the main source of contamination of pasture in the spring (13, 27).

A study conducted in our country highlights the role of lambs as a possible source of zoonotic infection when they are infested of another protozoan, *Cryptosporidium parvum* (16).

In France (2020), the most widespread species of *Cryptosporidium spp.*

identified were: *C. parvum*, *C. xiaoi*, and *C. ubiquitum*. In this study, the zoonotic species is *Cryptosporidium parvum* and highlights the potential role of lambs and sheep as a source of infection and potential zoonotic reservoir for human infections (6).

Several sheep farms and surface waters surrounding sheep farms in Greece (2020) highlight the importance of cryptosporidiosis and giardiasis due to their zoonotic potential and impact on human health, often causing outbreaks of waterborne diseases. The most prevalent species identified in both water and animal samples were: *C. parvum* (75%) and *G. duodenalis* (69%) (19).

The main gastrointestinal endoparasitosis diagnosed in a sheep flock from Brazil (2021) were: eimeriosis 36.2%, cestodosis 8.81% and strongilidosis 64.25% (9).

The treatment of infected lambs is a concern of veterinarians and a research topic found in studies both nationally and internationally. Thus, the unanimous opinion is that Toltrazuril, administered alone or in combination with other substances (lasalocid), is an important candidate in the fight against *Eimeria spp.* (10, 14, 29).

In the present study, we chose Toltrazuril and Diclazuril, respectively, in the therapeutic protocol, which were found to be effective against *Eimeria spp*., with a significant decrease in E.P.G., with Baycox (Toltrazuril) ascribed a higher efficacy.

In agreement with our results, join the findings realized by Le Sueur et al. and confirmed by Diaferia et al. (12) who support the superior therapeutic efficacy of Toltrazuril over Diclazuril for natural infestations in pasture-raised lambs (12, 18, 23).

Parasitism with ruminant cestodes requires infestation through consumption of grass by domestic livestock. After proglottis contamination, pasture is contaminated with infested elements after about 3 months (15).

Because of their low distribution, intermediate hosts play a little role in spreading parasites to other pastures. Lambs are more affected than adult sheep, with disease developing clinically after grazing (3, 17).

Expert recommendations for cestodicidal therapy of infected lambs begin with niclosamide and continue with the nematodicides fenbenedazole, oxfendazole, and albendazole and the fascio-licides bitionol and bitionol oxide, which also have cestodicidal activity (1, 4, 5, 7, 24, 28).

The results of the present therapeutic study show cestodicidal activity superior to Praziquantel over Albendazole. Statistical analysis of the results using the Kruskal-Wallis test indicates a significant decrease in E.P.G. when the protocol was administered with praziquantel+toltrazuril.

Conclusions

Oocysts of protozoa and oviger proglots (cestodes oncosfers) were identified by coprological examination in lambs from a farm in Valcea County, Vaideeni village.

Necropsy examination revealed the presence of *Moniezia expansa* in the small intestine.

The descriptive statistics associated with the scores corresponding to the E.P.G. obtained by the coprological examination and the application of the Kruskal Wallis test to determine the effectiveness of the treatment support the recommendation of the combination of Praziquantel (Prazicest) and Toltrazuril (Baycox 5%) in lambs infested with *Eimeria spp*. and tapeworms

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TICK FAUNA OF CATTLE IN NORTHEASTERN PART OF SERBIA (BANAT)

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Summary

The present study was conducted in 30 cattle herd from the territory of Banat (Vojvodina) in the period of March to October 2019, during the grazing season was to established tick fauna in that arae. Ticks were collected from cattle and from pastures where they were grazed. The tick species and sex/gender were identified by morphometric characteristics. Relative abundance analysis revealed that the *I. ricinus* was absolutely dominant species found in 71.22%, followed by *Haemaphysalis punctata* (18.22%), *Dermacentor marginatus* (11.72%), *Rhipicephalus sanguineus* (3.22%) and *Rhipicephalus bursa* (2.01%). On the pasture, however, the most common species was *H. punctata,* followed by *I. ricinus, D. marginatus, R. sanguineus* and *R.bursa.* Out of the total number of ticks collected, 53.65% were females and 46.35% were males. The sex ratio showed a higher number of females in four species (*Ixodes ricinus, Haemaphysalis punctata, Rhipicephalus sanguineus* and *Dermacentor marginatus),* while higher number of males were detected in *Rhipicephalus bursa.* The population dynamics of recorded tick species showed two annual maxima, in spring (April-May) and in autumn (September-October). The considerable interchange between spring and autumn tick populations can be attributed mainly to environmental conditions.

Keywords: cattle, ticks, Banat, Serbia.

Northeastern part of Serbia**,** Banat is part of Vojvodina, and is limited by the flows of the Tisza in the west, the Danube in the south, the Serbian-Hungarian border in the north and the Serbian-Romanian border in the east. The relief of Banat consists of several morphological units: Vršac Mountains (641 m above sea level), include Gudurički vrh, the highest elevation of Vojvodina. Belocrkvanska kotlina, is located south of Vršac. The East Banat valley is a lowland between two large dunes, stretching north of Vrsac, all the way to Timisoara, it is a shallow valley lowered along faults on whose higher sides the first sand accumulative forms began to form, the forerunner of today's Deliblato sands. Deliblato (Great) sandstone, is one of the largest European sand accumulations formed after the disappearance of the

Pannonian Sea. The sandstone is cultivated, forested, and its sand is frozen so that the wind would not blow it away and thus change the relief. Banat sandstone is located in southeastern Banat, stretches from southeast to northwest for almost 60 km, and is bordered by the Danube plain in the southeast and the Tamis in the northwest. The altitudes of the sandstone vary between 120 and 240 m above sea level, with an area of about 300 square kilometers. The sandstone in the narrow sense has a length of 38 km and a width of 11 km. The Banat light plateau surrounds the Banat sandstone and represents the accumulation of light dust, finer and finer sand material, which due to its lower weight is carried away from the sand and deposited where the transport power of the wind has weakened.

Some parts of Banat are abundant in grasslands where animals grazing is mainly semi-intensive. The rational use of pastures in the period of April-October makes the cattle production sustainable and low input in this period of the year. The specific climate and the unique habitat includes high biodiversity of flora and fauna of the grasslands. Pastures are not treated with insecticides, and usually, cattle share the pastures with other animal species depending on the location (e.g., domestic animals such as sheep, goats, horse, dogs, and wildlife species such as foxes, wild boars, several species of deer, and rodents) (3, 25, 29).

Ticks represents one of the indispensable elements of that biotope and tick infestations are common, especially during late spring and autumn months of the year. However, in the semi-intensive breeding system, which is the most often practice in this region, is very difficult to avoid infections with different types of ticks that are contaminants of the grasslands and pastures (25). Ticks are obligate haematophagous ectoparasites which have multiple adverse effects on the host organism. A particular problem is that they spread diseases to humans, domestic and wild animals, which can be reservoirs, vectors and/or transient hosts for the tick-borne pathogens (8, 9, 19, 22, 26, 29).

The tick fauna is so far the most studied in small ruminants in Serbia (27) and this is a continuation of research focused on cattle in some regions of Serbia. In our paper we presented results of examination performed during 2019 at northeast part of Serbia in Banat area (Vojvodina).

Materials and methods

During our examination we examined 177 cattle from 31 herds. The ticks present on animals were collected manually by removing them from cattle with tweezers and were placed in vials with 70% ethanol. From pastures ticks were collected by the flagging method using 1 m^2 white linen. Ticks were collected at the center of the pasture as well as under scattered vegetation present at the locations.

The tick species and sex/gender were identified by morphometric characteristics. The main attribute of identification of tick family is a plain dorsal sclerotised scutum or shield, which is often ornate with patterns in white or gold against a brown or grey background and which distinguishes these ticks from other

families. This sclerotised plate covers the entire dorsal surface of the male, but only one third of the female's dorsal surface. Second one was the capitulum of hard ticks which just as the mouthparts and is visible from a dorsal view. The peritreme or groove is big and clearly visibly around the stigmal plate. Grooves are deep, linear depressions in the body cuticle, usually on the ventral surface. Hard ticks can be easily differentiated by the shape of the basis capitulum and by the form of anal grooves (10, 11).

Results and discussions

During our examination a total of 326 ticks were collected from the 78 animals (44.06%) and a total of 87 ticks were collected from the pastures. We found only adult ticks. Relative abundance analysis revealed that the *Ixodes ricinus* was absolutely dominant species found in 71.22%. Followed by *Haemaphysalis punctata* (18.22%), *Dermacentor marginatus* (11.72%), *Rhipicephalus sanguineus* (3.22%) and *Rhipicephalus bursa* (1.30%) (Fig. 1).

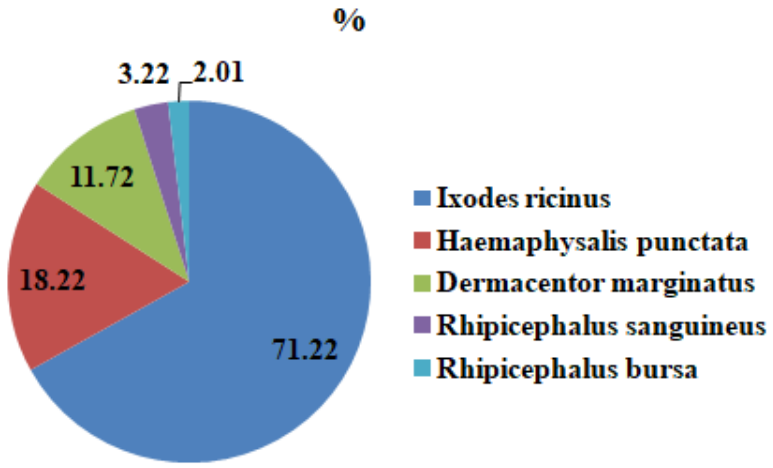

Fig. 1. Prevalence of tick in cattle in North Banat

On the pasture, however, the most common species was *H. punctata* found in 44.45%, followed by *I. ricinus* (42.85%), *D. marginatus* (8.55%), *R. sanguineus* (2.85%) and *R. bursa* (1.60%) (Fig. 2).

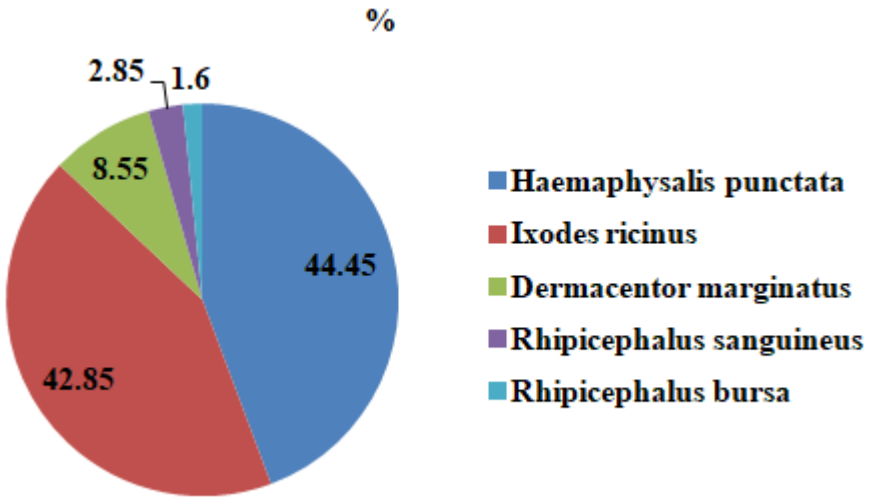

Fig. 2. Prevalence of tick in pasture in North Banat

Out of the total number of ticks collected, 53.65% were females and 46.35% were males. The sex ratio showed a higher number of females in four species *I. ricinus, H. punctata, R. sanguineus* and *D. marginatus,* while higher number of males were detected in *R. bursa.*

Climate condition like air temperature, relative humidity and rainfall and climate conditions have a great influence on the population dynamics of ticks. (1, 2, 4, 5, 6, 15). In the Banat the climate is moderate continental. The average annual temperature is 10.9ºC. The average winter temperature is -1ºC and in July is 21.6ºC. Annual rainfall is 686 mm, with 122 rainy days. The lowest point of the Danube is 70.83 m and the highest is 79.70 m above sea level. The highest recorded water level is +778 cm and the lowest is -134 cm. The influence at climate condition to population dynamics of ticks was monitored from March to October. They showed two annual maxima, in spring (April-May) and in autumn (September-October). The March was a period when the grazing season started and the first occurrence of *Ixodes ricinus, H. punctata* and *D. marginatus* was recorded. Two species *D. marginatus* and *H. punctata* occurred population maximum in April. May was the month of the population peak for *I. ricinus* and it was noted that this species started to decrease in abundance in June. *R. sanguineus* and *R.bursa* reached their maxima decreasing gradually until August, and disappearing completely in Septembar and October. The autumn population peak in Septembar and in October occurred for the *I. ricinus, D. marginatus* and *H. punctata.* Our results confirmed the results of the similarly studies carried out in northeast, eastern and south-eastern part Serbia (15, 16, 17, 18, 27).

The female abundance of established tick species has been in correlation with previously established population dynamics. The females of *Ixodes ricinus* species were present from March to October, with a peak population in May and June. Females of two species of the genus *Rhipicephalus* (*sanguineus* and *bursa*) have been found most often in the summer months - June and July. Findings of the females of species *D. marginatus* and *H. punctata* were most common in April and May. This population dynamics of female ticks is characteristic for this microclimate (2, 7, 13).

Males of the species *I. ricinus* were found from March to October, with the spring peak population in May and autumnal in September which corresponds to the values obtained in our earlier research (8, 26). Males of the species *R. sanguineus* were established from March to October, a species *R. bursa* from April to September with a population peak of both species in June, which also corresponds to values for this geographical area (28, 30, 32, 38). Males of the two species of the genus *Dermacentor* (*marginatus* and *pictus*) were usually found from April to June while the males of *H. punctata* species were established from April to June which corresponds to values of research in this area and in Central Europe and the Mediterranean basin (9, 12, 14, 18, 20, 21, 23, 28).

Conclusions

The found species of ticks are most common in ruminants in other coutries in the Western Balkan – North Macedonia, Montenegro and Bosnia and Hercegovina and in Hungary and Romania. A diverse tick fauna present in this region mainly influences the health status of grazing ruminants. Being the vectors and reservoirs for many tickborne pathogens like *Anaplasma phagocytophilu, Babesia divergens* etc., the tick transmit diseases that cause health disturbance in domestic animals and humans in affected areas. From that reason, the tick population need to be studied in order to predict the critical points and implement adequate protection measures in animals with the final goal of disease prevention and control.

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IDENTITY OF ISOLATED STRAINS OF ADENITIS EQUI STREPTOCOCCUS IN KAZAKHSTAN WITH EUROPEAN STRAINS

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Summary

Horse breeding in Kazakhstan is a traditional industry and, according to the Ministry of Agriculture of Kazakhstan, in June 2021, there are up to 2.9 million horses. The intensive development of horse breeding highlights measures to combat the factors that hinder the development of this industry. One of these factors is an infectious disease - *Adenitis equorum*. It is an acute, contagious disease of horses, mainly foals, characterized by fever, catarrhal-purulent inflammation of the mucosa, nasopharynx, and lesions of the regional lymph nodes (submandibular, pharyngeal, and other regions). The incidence of the disease varies from 3-5% to 70%. Mortality is 1-7%. The present research describes obtaining, for the first time, an inactivated, intranasally administered vaccine, which will be developed from purified antigens containing extracellular proteins, the interaction of which with the epithelial cells in the nasal cavity of animals is one of the main factors in the growth of immunogenicity. The analysis of the genomes of the causative agent of washing horses will be performed using data from the literature and nucleotide sequence banks. Selection of primers and probes for conserved regions of the genome using computer programs. The research activity involves the development and introduction into veterinary practice of an inactivated vaccine against *Adenitis equorum* for intranasal use. The results of this work will make it possible to optimize anti-epizootic measures for the prevention and spread of this disease among horses.

Keywords: *Streptococcus equi*, *Adenitis equorum*, strangles.

Strangles is an infectious disease caused by *Streptococcus equi subsp equi*. The disease has an acute evolution characterized by fever, catarrhal-purulent inflammation of the mucous membranes of the nasal cavity, pharynx, and adsorption of submandibular lymph nodes.

Bacteria located in the nasal cavity and guttural pouches of infected animals play an important role in spreading the infection.

The international transport of horses, competitions, races, and trade in horses could promote the spread of pathogenic strains of staphylococci and streptococci, both to other animals and to humans (17, 18).

The disease mainly affects the young horse population in the autumn-winter-spring period of the year. The foals usually get sick on weaning, but they can also get sick at the age of one month or ten days.

The disease is transmitted through drops in the air, direct contact,

contaminated feed, and water (17).

When the foals are weakened due to the poor nutrition of crowded horses, the onset of the disease is imminent. In view of the difficult epizootic situation in the strangles of horses in the Republic of Kazakhstan, were collected samples of pathological material from the horses with clinical signs.

The aim of the paper was to isolate the bacteria of *Streptococcus equi* subsp *equi* and to study the morphological and cultural-biochemical and molecular-genetic properties of this bacterium for later use in the development of preventive and therapeutic preparations against horse strangulation. Isolated *Streptococcus equi subsp equi* samples were compared with European strains.

Materials and methods

The laboratory examination was performed on pathological materials (nasal swabs, abscess aspirates, or guttural pouch lavages) from horses with clinical signs. Bacteriological and bacterioscopic examinations were performed, and final identification by PCR using the commercial kit QIAGEN.

Smears prepared from pathological material, as well as from broth and agar cultures, were stained by Gram (2).

The identification of the culture isolated from *Streptococcus equi* was performed by studying the biochemical properties - by isolating the enzymatic saccharolytic, proteolytic enzymes, the formation of catalase, oxidase, and ammonia, hydrogen sulfide, and indole (4, 5, 12).

The sensitivity of isolated isolates to antibiotics was determined using antibacterial disks. The following antibiotics were used for this: Gentamicin, Penicillin, Streptomycin, Tetracycline, Amoxicillin, Ciprofloxacin, Metronidazole, and Trimethoprim.

Confirmation of the molecular-genetic properties of *Streptococcus equi* isolate was performed using PCR analysis. Bacterial DNA isolation of the studied isolate was performed using a commercial QIAGEN kit. A Gene Atp PCR amplifier was used to amplify isolated DNA (7).

Results and discussions

Streptococcus equi strains were isolated from the pathological materials studied. Bacterioscopic examination revealed the presence of gram-positive streptococci in abscesses, tampons of the nasal and oral cavities, and tampons of the conjunctival mucosa.

Bacterioscopy revealed the presence of short coccoid gram-positive bacteria in the form of twisted chains, slightly flattened in diameter.

In the bacterial cultures isolated from pathological material from a foal, it was found that the isolated streptococcus does not ferment lactose, sorbitol, mannitol, does not form indole, ammonia, H_2S, litmus, and methylene blue does not reduce,

does not dilute gelatin, does not coagulate sterile skimmed milk.

At the same time, biochemical properties showed that the studied isolate is oxidase negative and catalase positive. The absence of fermentation of these carbohydrates makes it possible to differentiate the *Streptococcus equi* from the pyogenic one *Streptococcus pyogenes*.

The following antibiotics were used for the antibiogram: Gentamicin, Penicillin, Streptomycin, Tetracycline, Amoxicillin, Ciprofloxacin, Metronidazole and Trimethoprim.

As a result of the research, it was found that the isolate is sensitive to amoxicillin, gentamicin, and ciprofloxacin. The inhibition area of the non-growth of bacteria on the above antibiotics exceeded 30 mm. Less sensitive to streptomycin, tetracycline, and trimethoprim. Resistant to penicillin and metronidazole.

Some authors thus suggest a possible use of cephalosporins (ceftiofur), macrolides (erythromycin), ampicillin, trimethoprim sulphonamide, and oxytetracycline (2).

Other authors found that most *Strept. equi* sub. sp. *equi* more sensitive to ampicillin, streptomycin, tetracycline, and gentamicin and less sensitive to erythromycin, kanamycin, and lincomycin, these antibiotics were recommended in treating clinical cases (1, 5, 19).

During an outbreak, prompt initiation (within 24 hours) of antibiotic therapy on new cases in the early acute phase presenting with fever, depression, anorexia, or discharge may be curative and avoid local abscess formation (11, 19, 20).

Their effectiveness is good because in the absence of abscesses they can reach the bacteria directly.

The major etiological diagnosis is based on the demonstration of the pathogen in various samples: nasopharyngeal swabs, nasal wash, throat bag wash, pus samples, or purulent discharge. Two methods are possible for this: bacteriology and PCR.

Therefore, rapid identification of *Streptococcus equi* is a high priority for the clinician.

Identification of asymptomatic carriers falls under offensive and defensive prophylaxis. This aspect of prophylaxis is all the more important as it appears that more than 75% of strangle outbreaks result in one or more asymptomatic carriers.

Bacteriology: culture and isolation of bacteria on Columbia blood agar followed by serogroup determination (Lancefield classification). Finally, by testing its saccharide fermentation capabilities, it is possible to identify the bacterium with certainty (6, 13, 15).

Currently, the reference technique for the diagnosis of strangles is bacteriological analysis.

In one study, of the 25 horses declared affected by strangles, all had a positive PCR and bacteriological result, except for one that was PCR positive and bacteriologically negative and 4 for which PCR could not be performed, due to lack of material biological material provided for analysis (7, 16, 21).

PCR analysis confirmed the molecular-genetic properties of *Streptococcus equi* isolates. Bacterial DNA isolation of the studied strains was performed using a commercial QIAGEN kit.

The nucleotide sequence of the bacterial strains of *Streptococcus equi* was identical to the nucleotide sequence of the 16 S rRNA gene fragment of *Streptococcus equi*. The bacterial isolate belongs to the species *Streptococcus*, the equivalent of the behavior similar to the European strains.

PCR (Polymerase Chain Reaction) is used to look for the presence of the bacterium by highlighting the amplified genomic sequences. In the case of strangles, the gene sought is that of the M protein. This technique is much more sensitive than bacteriology, but it is also less specific because it does not make it possible to differentiate between dead and living bacteria. It is the technique of choice for searching for asymptomatic carriers, monitoring their treatment, and diagnosing the condition of an animal for import or export (7, 16, 21, 22).

There are different types of vaccines (inactivated, containing the M protein) with variable, but so far not optimal, effectiveness. The intramuscular administration method does not make it possible to induce the local immune reaction mainly involved in immunized horses, sometimes at the origin of the phenomenon of local intolerance (3, 10).

The most recently introduced on the market is a live attenuated vaccine that is administered submucosally in the upper lip, with a booster every three to six months depending on the infection pressure. It is possible to carry out an emergency vaccination in already vaccinated horses during an episode of strangles (5).

Outbreaks of strangles are common in many countries and negatively affect the health of horses and cause major economic losses to the equine industry worldwide (1, 8, 9).

The surveillance of outbreaks as well as the testing and treating of strangles carriers can reach disease control and prevention and will lead to the break of the cycle of infection, and, eventually, to disease eradication.

Conclusions

Cultures of *Streptococcus equi* isolated in terms of cultural and morphological properties corresponding to the causative agent of strangles.

The rapid test provides a valuable aid in the identification of group C beta-hemolytic streptococci in an efficient and cost-competitive manner.

The results of molecular genetic testing also confirm that the isolated isolates belong to the species *Streptococcus egui subsp equi.*

*Streptococcus equi subs equ*i strains will be the basis for the preparation of an inactivated intranasal vaccine in horses with strangles**.**

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THE ECONOMIC SIGNIFICANCE OF DECONTAMINATION IN SWINE MATERNITIES

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Summary

Depopulation/repopulation interval and decontamination are too often ignored in the intensive swine production based on so-called economic reasons. The present study aims to combat this idea with technical and economic arguments, by analyzing the farrowing records from a breeding farm with 6000 farrowings monthly, from August 2019 to February 2020 (7 months). The following indicators were analyzed: total born piglets, from which live-borns, stillborns, mummified, weaned and average weaning weight. From September 2019, all the decontamination steps were followed, and starting from January 2020 the decontamination of the sows' body was also performed. The average values of the indicators, for each month, were the following: total born piglets - 15.93 (August), 16.29 (September), 16.38 (October), 16.39 (November), 16.86 (December), 17.56 (January) and 17.63 (February); live-born piglets - 14.23, 14.96, 15.20, 15.37, 15.53, 15.71 and 15.63; the average number of weaned piglets per sow - 10.68, 10.94, 11.12, 11.52, 11.34, 11.65 and 11.52; average weaning weight (kg) - 5, 5.4, 5.9, 6, 6.2, 5.9, 6.2. It was found that surfaces and sows' body decontamination have both technologic and economic advantages.

Keywords: swine maternity, decontamination, economic impact.

Intensive industrial animal husbandry determines the reassessment of the ratio between humans, animals and the environment, in order to be able to provide quantitatively and qualitatively the protein necessary for the entire population of the world while meeting the welfare requirements of the animals and simultaneously with the protection of the environment at both micro and macroeconomic level (4, 7, 8).

Within the microorganism species, the sensitivity to physical and chemical agents of germs in a population is not uniform. For example, the foot-and-mouth disease virus is fully inactivated at pH=4 within a few seconds, and at pH=5 in two minutes, but parts per million (ppm) viruses resist under these conditions for 30 minutes (2, 3). In the pig farm industry, water is a major problem, because animal husbandry generally consumes 33% of the fresh water used by man (10, 11, 15, 16). The transition to the market economy, the privatization of the livestock and food industry units, on the one hand, and the liberalization of the veterinary medical practice, on the other hand, as a result of Romania's accession to the European Union, have led to significant changes in the strategy and tactics of preventing and combating diseases, both in animals and in humans, which have led to the transfer of responsibility from the state to farmers, who are economically interested in raising healthy animals and producing food without infectious risk, and are now also legally

responsible for animal health management and the protection of public health (12, 13, 14). It is mandatory to thoroughly master and observe the principles of biosecurity in livestock farms (1). Recent study shows that if there is 465000 CFU/cm^2 on a surface, by washing with plain water the microbial load is reduced to about 77500 CFU, and if the washing is done with detergent solutions at 15500 CFU/cm^2, so that by disinfection it decreases to 155 CFU/cm^2 (17).

There is a wide variety of devices for the dispersion of disinfectants. Some are with manual drive and others with electric drive. For many devices, the dosing of antimicrobial substances is done automatically, resulting in dilution at predetermined concentrations (0.25 – 10%) (5, 19). The effect of washing is not limited only to the removal of dirt, but to some extent also causes a reduction in the degree of microbial contamination (9). Chemical agents indicated for use in cleaning surfaces, which come into contact with foodstuffs, must meet a number of requirements, namely: be non-toxic and non-corrosive; be able to emulsify and saponify fatty substances; do not precipitate the calcium and magnesium salts in the water and keep the dirt particles in suspension (6, 18, 20).

Materials and methods

The study was carried out in a breeding swine farm. The farm has 20 halls of which seven maternity halls and 13 breeding halls, which in turn have a hall for mating and acclimatization of gilts, two halls for early gestation gilts, two halls for early gestation sows and eight halls for advanced gestation.

In the maternity hall after mechanical cleaning of the speaker, a detergent-based solution was applied. After applying the detergent with the help of industrial washing pumps with high working pressure (200 bar), the actual hidric sanitization was performed. After a careful check and removal of any residues from the surface thus prepared, the disinfectant solution was applied. After applying the disinfectant, the hall was closed and left empty for 24 hours. 24 hours after the disinfection, the hall was ventilated and sanitizing probes were collected to check the efficiency of disinfection.

Upon confirmation of the results by the laboratory that the samples were negative, other animals were introduced into the farrowing boxes with the mention that before their entry, the sows were washed, paying great attention to the udder, the posterior area, the legs but not only this ones, all this so that at the end of the action the animals no longer present manure residues on the skin, only now a disinfectant was applied to the body of the sows that were going to suckle in 5-10 days. Until the movement in the farrowing crates, a thin layer of calcium carbonate (whitewashed) with a special pump was applied as an additional measure.

Results and discussions

The study was carried out on the maternity halls for a period of seven months, the first four months representing the period when the disinfection and sanitization of the shelters was carried out poorly (control group), and the next 4 months the disinfection was carried out rigorously (the experimental group).

In total, the data were obtained following calving and weaning, and the indicators followed were the following: totally calving piglets; dead farrowing piglets; crushed piglets; viable farrowing piglets; piglets born alive and alive after 24 hours; weaned piglets on the sow; weaned piglets per month; days of weaning life; mortality of piglets; kilograms when weaning piglets.

The analysis of the results reveals that immediately after farrowing we obtain data from the following indicators: the number of fully calving piglets, viable calving piglets and live and alive piglets after 24 hours were higher in the study group and the number of dead calving piglets and the number of crushed piglets in the first 24 hours was lower in the study group.

After weaning the sows and delivering the piglets, we obtain the following data: the number of piglets that reached weaning, the average of the weaned piglets per month and the weaning weight of the piglets was higher in the experimental batch and the mortality of the piglets from calving to weaning was lower in the study batch.

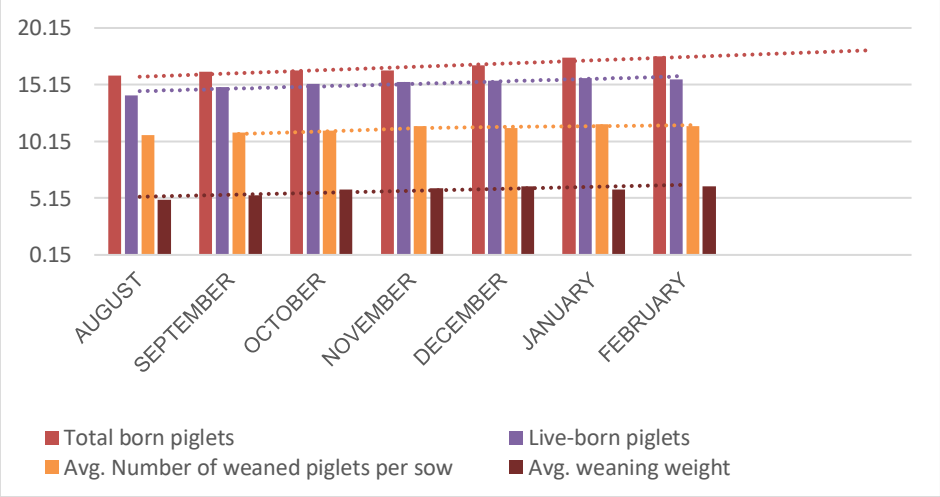

Fig. 1. Parameters values for each month

The average values of the indicators, for each month, were the following: total born piglets - 15.93 (August), 16.29 (September), 16.38 (October), 16.39 (November), 16.86 (December), 17.56 (January) and 17.63 (February); live-born

piglets - 14.23, 14.96, 15.20, 15.37, 15.53, 15.71 and 15.63; the average number of weaned piglets per sow - 10.68, 10.94, 11.12, 11.52, 11.34, 11.65 and 11.52; average weaning weight (kg) - 5, 5.4, 5.9, 6, 6.2, 5.9, 6.2 as presented in Fig. 1.

Conclusions

Proper washing and disinfection of the maternity box is a very important stage for the health and immune status of the future piglet but also a process that brings benefits, the weaning of more piglets with a lower mortality at a higher weight and the losses in the first 24 hours after calving are lower if the disinfection process and stages are rigorously observed and implemented.

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