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DIAGNOSTICS OF THE HEART CONDITION IN UKRAINIAN RIDING HORSES WITH A PORTABLE ECG MONITOR

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Abstract. *Electrocardiographic reference values of clinically healthy horses are needed for assessment of the reduced performance or cardiac pathology animals. The purpose of this study was to establish the rate of occurrence of arrhythmias in clinically healthy horses of the Ukrainian riding breed at rest, during exercises, and the recovery period. The experimental group of animals included 36 horses of age 3–17 years, 16 mares (adult female horses), 11 geldings (castrated males), and 9 stallions (adult males). The electrodes were placed with a modified base-apex system on the surface of animals' bodies. ECG recording was performed at rest (15 minutes), during exercises and the recovery period for 30–40 and 15 minutes, respectively. Prevalence and frequency of occurrence of arrhythmias were analysed with obtained recordings. A significant number of the second-degree atrioventricular blocks were detected in 13.9% cases (n = 5) during the recovery period. 33.3% (n = 2), 8.3% (n = 3), and 19.4% (n = 7) of cases of supraventricular premature contractions were detected during exercises, at rest, and during the recovery period, respectively. The incidence of supraventricular premature contractions was < 3/animal and < 5/animal during exercises, and recovery period, respectively; with the exception of one animal in which 9 and 7 premature contractions were detected, respectively. Ventricular premature contractions were identified in 2.7% (n = 1) and 5.6% (n = 2) cases, at rest and exercises, respectively. Supraventricular tachycardia with 6–9 consecutive premature contractions during trot was detected in 1 horse (2.7%). The results of this study have confirmed the presence of arrhythmias in clinically healthy horses. The type of arrhythmias significantly depends on the period (rest, exercises, and recovery) of the animal's activity, particularly.*

Keywords: *arrhythmias, heart condition diagnostics, portable ECG monitor, ECG records, horses*

Introduction

Electrocardiography is an indispensable and highly accurate method for diagnosing heart pathology, including arrhythmias. Any disorder in the cardiac impulse generation or conduction, changing the heart rhythm, causes an arrhythmia that can disrupt peripheral circulation. Electrocardiography is the most important method for diagnostics of arrhythmias (Alidady et al., 2002).

Arrhythmias are divided into two types: functional and organic. Arrhythmias of the first type are not associated with heart pathology, also called physiological or inorganic. For example, in horses and cattle, such arrhythmias can occur under the high influence of the autonomic nervous system. The second type, organic or pathological arrhythmias, is associated with heart disease or structural disorder in the cardiac conduction system (Dezfuli et al., 2001).

The value of electrocardiogram (ECG) in horses at rest is limited because most of the arrhythmias have a latent course and can occur only during the exercise. Furthermore, some disturbances in cardiac rhythm may appear after exercise during the recovery period (Young, 2007). In this case, for the complete diagnosis the ECG should be recorded not only at rest, but also during and after the exercise, especially in horses with a history of collapse.

Analysis of recent researches and publications

In 1911, Lewis recorded the first case of a pathological ECG of a horse with atrial fibrillation (Lewis, 1911). Since then many researchers, both foreign and domestic ones, have studied arrhythmias in horses.

Borovkov et al. (2013) studied the effect of age on the condition of the cardiovascular system in Ukrainian riding horses. They found that horses over 18 years old were more likely to have cardiac arrhythmias, namely sinoatrial and atrioventricular (AV) blocks.

Maksymovych studied the prevalence and significance of arrhythmias before and after exercise in athletic horses of Ukrainian riding, Hanoverian and Westphalian breeds. He reported that sinus arrhythmia (SA), second-degree AV block, and supraventricular premature complexes (SVPCs) were diagnosed with a high prevalence (Maksymovych, 2016).

Ryan et al. examined arrhythmias in apparently healthy Thoroughbred horses during and after exercises. The rate and prevalence of such arrhythmias as sinus arrhythmia, second-degree AV block, SVPCs, and ventricular premature complexes (VPCs) were established in their research (Ryan et al., 2005). Another research conducted by Buhl et al. investigated prevalence of arrhythmias before, during, and after exercises in clinically healthy show jumping horses of different breeds (Buhl et al., 2010).

The Ukrainian Riding Horse, or Ukrainian Saddle Horse, also known as Ukrainian Warmblood, is a relatively young Ukrainian breed of warmblood sport horses. Breeding of these horses has begun since 1945 and the genetic base constituted Hanoverian, Thoroughbred, and Trakehner stallions with local mares or Hungarian Furioso, Gidran Arab, or Nonius mares (Edwards, 2016). It was bred as show jumping, dressage and three-day eventing breed, but also suitable as a general riding horse (Rousseau et al., 2017).

Due to the lack of literature describing cardiac arrhythmias in Ukrainian riding horses during the exercises, the

clinical significance of heart rhythm disturbances is yet to be established.

Purpose. The aim of this study was to document the incidence of arrhythmias in clinically healthy horses of the Ukrainian riding breed. The electrocardiography was performed in horses at rest, during exercises and the recovery period.

Materials and methods of research

The study was conducted in equestrian sport complexes and private stables in Kyiv region of Ukraine ("Kentaur", "Pavlovsky Passage", "Rodeo", "KSK Koenig", "Kyivska Rus", and others).

In order to confirm the clinically healthy condition of the animals and to form the experimental group, a physical examination was carried out, which included: anamnesis collection, rectal temperature measurement, examination of visible mucous membranes (conjunctiva of the eyes), determination of capillary refill time, apex beat (precordial area), arterial pulse (maxillary and digital arteries), and cardiac auscultation. Animals with clinical signs of oedema and the presence of heart murmurs were not included in the experimental group.

Animals. The experimental group of animals have included 36 horses at age of 3–17 years, 16 mares (adult females), 11 geldings (castrated males), and 9 stallions (adult males). 10 horses competed in dressage, 9 – in show jumping, 17 – did not take part in competitions, but 3–4 times a week had a general lungeing training.

ECG recording. The ECG registration in Ukrainian riding horses was performed using a portable ECG monitor [a portable ECG monitor developed by scientists of the National Technical University of Ukraine "Kyiv Polytechnic Institute named after Igor Sikorsky"]. With this purpose the system of 4 electrodes was placed as follows: the negative (red) electrode was attached on the right in front of the scapula, the positive (green) one – on the left directly behind the area of apex beat caudally from the elbow, the positive-negative (yellow) one – 5–10 cm above the green, and the reference (black) electrode – on the left in the area of the scapula (Popadiuk & Holopura, 2020). Disposable electrode pads with a gel layer and a sticky base [SKINTACT F – 55, Innsbruck, Austria] were used. For better contact and stability of the electrodes during movement, a girth covering all electrodes was used. The ECG recordings were stored on a memory card of the device with the subsequent uploading to the cloud storage.

Exercises. The outdoor training was performed during spring and fall, in the morning with the temperature approximating to be 13–20 °C. ECG recording in horses was performed in three stages:

1) at rest before exercise for 10–15 minutes.

2) during physical activity for 30 – 40 minutes.

3) during the recovery period immediately after exercise for 15 minutes.

The stage of physical activity included the warm-up and the exercise itself. The training protocol consisted of 7 intervals: 2 at a walk, 2 in trot, 2 in canter and 1 in a gallop. Each of the intervals lasted 3–10 minutes with a distance of 2000–4000 meters.

ECG analysis. Arrhythmias diagnostics was based on the following principles:

- second-degree AV block: the absence of the QRS complex for the P wave on the interval between the two previous P-P intervals. There are two types distinguished:

physiological Mobitz type I with Wenckebach periods, when the PR interval gradually lengthens before the block, and pathological Mobitz type II, in which the P-R interval has a constant duration (Hilwig, 1977).
- sinus arrhythmia: variability of R-R intervals, in which the heart rate (HR) for several heartbeats decreases, increases, and decreases again, until a more stable rhythm is established (Menzies-Gow et al., 2001).
- sinus pause: increasing of the R-R interval by 20% at rest and by 10 % during exercise; the P waves have a normal configuration (Ulfberg & Clark, 2006).
- sinus block: the absence of the P wave for a time equal to the duration of the double previous P-P interval (Bonagura & Miller, 1985)
- SVPCs: decreasing of the P-P interval by 20 % at HR < 60 beats/min (bpm), by 10 % at HR of 60–100 bpm, and by 4 % at HR > 100 bpm; the morphology of the P wave may vary depending on the site of the impulse origin; premature contraction may be accompanied by a compensatory pause (Flethoj et al., 2016).
- supraventricular tachycardia (SVT): a series of three or more consecutive SVPCs.
- (VPCs: shortening of the R-R interval by more than 20%; there is no P wave before the QRS complex; the QRS complex has an abnormal configuration (Buhl et al., 2010).

Statistical analysis. Descriptive analysis included determining the prevalence and frequency of arrhythmias, as well as the mean and 95% confidence intervals for the HR at three periods of each individual (rest, exercises, and recovery). Statistic data analysis was performed by two-way analysis of variance (ANOVA) with such factors as arrhythmias and periods (rest, exercises, recovery) in the SAS software [SAS University Edition, NC, USA]. The results were considered significant at $P < 0.05$.

Results of the research and their discussion

The minimum and maximum HR for different periods (rest, exercises, and recovery) are shown in Fig. 1. The maximum HR during the gallop averaged 180–200 bpm.

ECG recordings (Fig. 2) are demonstrating sinus pause, sinus block, second-degree AV block, SVPCs, VPCs, and SVT. The prevalence and frequency of arrhythmias are shown in Table 1. The second-degree AV block was detected in 5 (13.9%) horses during the recovery period. The prevalence and frequency of SVPCs and VPCs in individuals are shown in Table 2. In 12 animals (33.3%) the SVPCs were detected during exercise, and in 3 (8.3%) and 7 (19.4 %) at rest and during the recovery period, respectively. The incidence of SVPCs was < 3/animal during exercise, and < 5/animal during the recovery, except for one animal, in which 9 and 7 premature contractions were detected, respectively. VPCs were identified in 2.7% (n = 1) and 5.6% (n = 2) cases at rest and exercises, respectively. SVT with 6–9 consecutive premature contractions during trot was detected in 1 horse (2.7%).

ANOVA included sinus pause, sinus arrhythmia, second-degree AV block, and SVPCs. The remaining arrhythmias were not included in the analysis because of the insufficient sample. A significant relation ($P < 0.0001$) between arrhythmias and different periods (rest, exercises, recovery) was found, con-

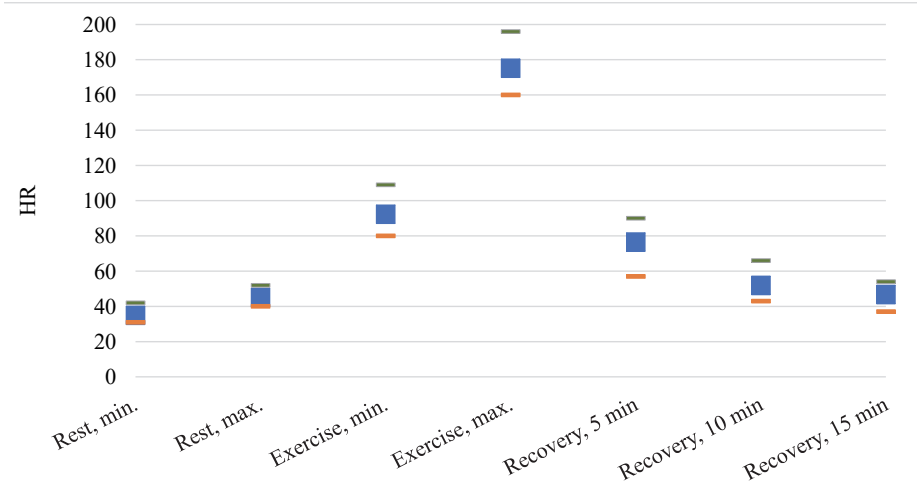
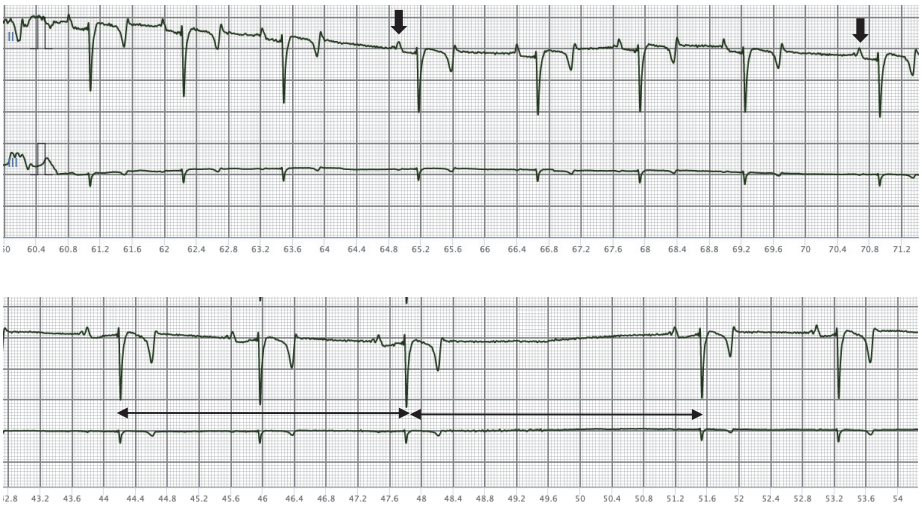


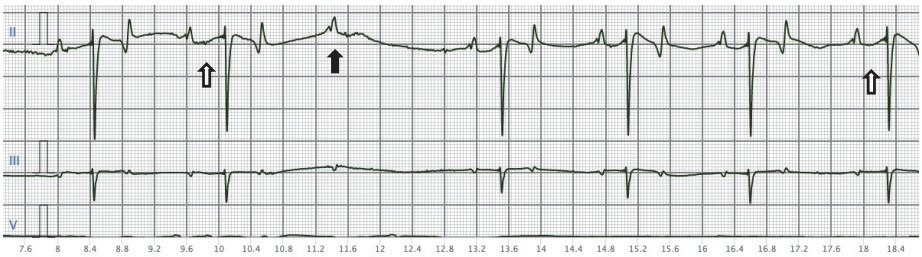
Fig. 1. The range of minimum, maximum values, and mean HR calculated for three periods (rest, exercises, and recovery, after 5, 10, and 15 minutes) of each individually. The error bars reflect the 95% confidence intervals

firming the fact that the type of arrhythmia varies depending on the period of activity or rest of the animal. During the exercise, an increase in the number of sinus arrhythmias ($P = 0.006$) and SVPCs ($P < 0.0001$), and during the recovery – an increase in the number of the second-degree AV block ($P < 0.0001$) was found.

A. Sinus pauses on the ECG of a 7-year-old gelding on a walk. The beat after the pause is marked with a filled arrow. HR = 50 bpm, Speed = 25 mm/s, 1 mV = 10 mm

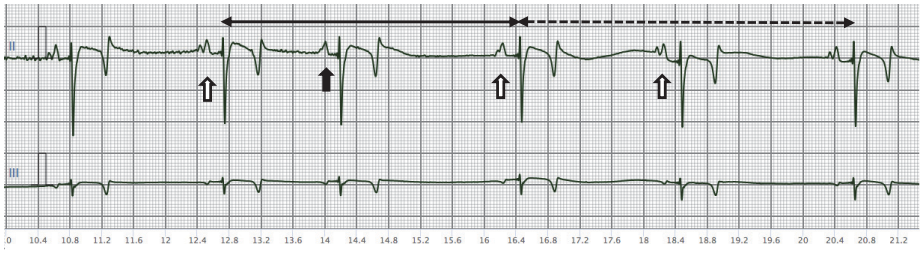
B. Sinus block on the ECG of a 5-year-old mare during the recovery period. The pause is equal to two R-R intervals. HR = 35 bpm, Speed = 25 mm/s, 1 mV = 10 mm





C. Second-degree AV block on the ECG of a 12-year-old mare during the recovery period. The blocked P wave is marked with a filled arrow. Some P-R intervals have a longer duration – Wenckebach phenomenon, marked with an empty arrow. HR = 31 bpm, Speed = 25 mm/s, 1 mV = 10 mm

E. VPCs on the ECG of a 5-year-old stallion at rest. The premature contraction (filled arrow) has a bizarre morphology, longer QRS duration, and is followed by a T wave with the opposite direction. The subsequent P wave (empty arrow) occurs in predefined time but is blocked and followed by a compensatory pause due to the refractoriness of the ventricular myocardium. HR = 39 bpm, Speed = 50 mm/s, 1 mV = 20 mm.



D. SVPCs on the ECG of a 7-year-old gelding at rest. The premature contractions (marked with a filled arrow) are followed by a noncompensatory pause, because the premature depolarization resets the sinus node, meaning that the R-R interval encompassing the SVPC (solid double arrow) is shorter than the normal R-R interval (dashed double arrow). Also, premature P wave has clearly different morphology compared to normal P waves (empty arrow). HR = 38 bpm, Speed = 25 mm/s, 1 mV = 10 mm

F. SVT on the ECG of a 9.5-year-old stallion during trotting. The atrial rate is about 75 bpm. The QRS complexes after premature P waves (marked with a filled arrow) show a larger amplitude and T waves have the opposite direction, which is often the case for short R-R interval. HR = 68 bpm, Speed = 25 mm/s, 1 mV = 10 mm.

Wenckebach phenomenon was present, so such arrhythmia was considered to be a physiological one.

Normally, horses may develop SVPCs both at rest and during the recovery. Frequent premature contractions during exercise, especially at the peak HR, are considered to be a consequence of atrial

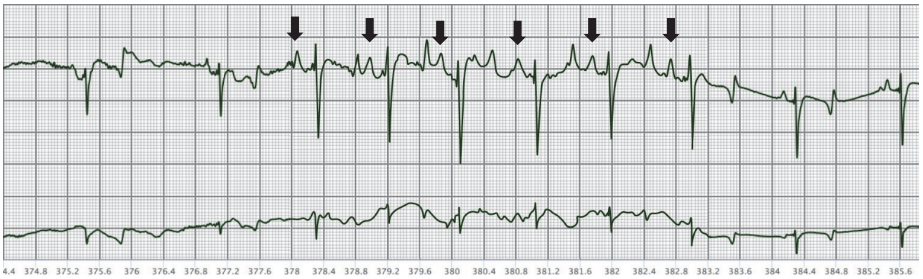


Fig. 2. ECG recordings with a sinus pause (A), sinus block (B), second-degree AV block (C), SVPCs (D), VPCs (E), and SVT (F)

For the first time, a detailed analysis of ECG for the presence of arrhythmias in Ukrainian riding horses before, during, and after the exercises was demonstrated.

Second-degree AV block, found in 6 animals (16.6%), completely disappeared during exercise and in 5 animals (13.9%) returned during the recovery period, when the HR decreased to the rest values. In all cases of diagnosis of this arrhythmia, the myocardial disease. The number of animals in which SVPCs were detected, as well as the frequency of extrasystoles per animal, was lower in our study compared to the data obtained by other researchers in their studies (Ryan et al., 2005; Buhl et al., 2010). Nonetheless, most SVPCs occurred during the low intensity of exercise and low HR, which is consistent with data obtained by other researchers. It is note-

1. Prevalence of detected cardiac arrhythmias in horses at rest, during exercises and the recovery period

Arrhythmia	Type of horse work		
	Rest (n,%)	Exercises (n,%)	Recovery (n,%)
Second-degree AV block	6 (16.6)	1 (2.7)	5 (13.9)
SVPCs	3 (8.3)	12 (33.3)	7 (19.4)
SVT	-	1 (2.7)	-
Sinus block	-	-	2 (5.6)
Sinus arrhythmia	4 (11.1)	10 (27.8)	6 (16.6)
VPCs	1 (2.7)	2 (5.6)	-
Sinus pause	2 (5.6)	3 (8.3)	5 (13.9)
Sinus tachycardia	1 (2.7)	-	1 (2.7)

worthy that no extrasystoles were detected during peak exercise, so its occurrence might be explained by the fluctuations in HR due to the changes in the pace and the influence of the autonomic nervous system on the equine heart.

VPCs are the result of a violation of electrical activity in the heart ventricles, resulting in premature contraction (Bonagura & Miller, 1985). The value of ventricular arrhythmias for animals often contradicts each other, but its occurrence is usually associated with myocardial disease (Reimer et al., 1992). Ventricular extrasystoles in the current study occurred at a higher HR (110–130 bpm) compared with SVPCs (45–90 bpm).

Single VPCs that occur at the end or immediately after the exercises, in the absence of structural myocardial disease, are considered to be physiological ones (Reef et al., 2014). In case if > 2 SVPCs or VPCs occur at peak loads, or > 5 pairs during the recovery period, its presence may cause poor performance, predispose to the development of ventricular tachycardia, and even collapse and sudden death (Martin et al., 2000). However, recent studies in clinically healthy horses, with significantly exceeding numbers of SVPs and VPCs, have questioned the validity of these cut-off values. The current study revealed fewer VPCs than in the studies of other authors, which may be explained by the different protocols of the exercise procedure (Ryan et al., 2005; Buhl et al., 2010; Maksymovych, 2016).

2. The number of SVPSs, VPCs, and SVT at rest, during recovery and exercises in horses, with periods specified

№	Age, years	Physiological condition of male and female	Rest and recovery		Exercises		
			SVPCs	VPCs	SVPCs	VPCs	SVT
1	4	M	3 rest	-	1 trot	-	-
2	5	S	-	1 rest	-	1 canter	-
3	5.5	M	2 rest, 7 recovery	-	5 walk, 4 trot	-	-
4	6	G	-	-	1 walk	-	-
5	7	M	-	-	1 walk	-	-
6	7	G	2 rest, 5 recovery	-	3 walk	-	-
7	7	S	-	-	2 trot	-	-
8	7.5	M	3 recovery	-	1 canter	-	-
9	8	M	2 recovery	-	1 trot	-	-
10	9	G	-	-	1 trot	-	-
11	9.5	S	-	-	-	-	2 trot
12	11	M	-	-	1 walk	1 canter	-
13	12	G	1 recovery	-	2 walk	-	-
14	13	M	1 rest, 1 recovery	-	2 trot	-	-

Note: M – mare, G – gelding, S – stallion.

SVT in most cases is pathological and occurs due to myocardial disease, atrial dilatation, or another congenital heart disease. Subsequently, SVT may lead to the development of atrial fibrillation (Marr & Bowen, 2010). In our study, it was detected in 1 animal and was manifested as 6 and 9 consecutive SVPCs.

The sinus pauses found in the current study have been previously described (Buhl et al., 2010). This arrhythmia was diagnosed during a walk or trot of the animal, and on the ECG, it is visually similar to a sinus block with a shorter duration. It can be assumed that sinus pauses occur during the return to vagal tone, resulting in a slight delay in the impulse conduction through the sinus node. Since the high vagal tone is typical for horses, the sinus pauses may be considered a physiological arrhythmia.

Conclusions and future perspectives of the study

As per our present study, it was clearly shown that usage of the portable ECG monitor confirms arrhythmias in clinically healthy horses of the Ukrainian riding breed. It was found that the type of arrhythmia significantly depends on the period (rest, exercises, and recovery) of their activity.

The second-degree atrioventricular block was most often (16.6%) registered at rest. During the exercises most frequently (33.3% of cases) supraventricular premature complexes develop, in particular, in most cases, premature contractions occur at a relatively low heart rate and low intensity of exercises.

The obtained reference values allow the veterinarians to assess the heart functions in horses of Ukrainian riding breed with poor performance, clinical signs of cardiac pathology, or cases of collapse in the case report.

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Анотація: *Електрокардіографічні референтні значення клінічно здорових коней є необхідними під час оцінки коней зі зниженою продуктивністю або серцевими патологіями. Метою цього дослідження було встановити частоту виникнення аритмій у клінічно здорових коней української верхової породи в стані спокою, під час фізичних навантажень та у період відпочинку. Дослідна група включала 36 коней української верхової породи віком від 3 до 17 років – 16 кобил (дорослі самки), 11 меринів (кастровані самці) та 9 жеребців (дорослі самці). Електроди розміщували за модифікованою осново-верхівковою системою на тілі тварин. Реєстрацію ЕКГ проводили у стані спокою (15 хв), під час навантажень та у період відновлення відповідно впродовж 30 – 40 та 15 хв. На одержаних записах аналізували переважання та частоту виникнення аритмій. Значну кількість атріовентрикулярних блокад у 13,9% (n = 5) було виявлено у період після навантаження. 33,3 % (n = 12), 8,3% (n = 3), 19,4% (n = 7) випадків суправентрикулярних екстрасистол було виявлено відповідно під час навантаження, у стані спокою та у період відновлення. Частота виникнення суправентрикулярних екстрасистол складала < 3/тварину та < 5/тварину відповідно під час навантаження та відновлення, окрім однієї тварини, у якої було виявлено відповідно 9 та 7 екстрасистол. Шлуночкові естрасистоли було виявлено у 2,7% (n = 1) та 5,6 % (n = 2) відповідно у стані спокою та під час руху. Суправентрикулярну тахікардію з 6 – 9 послідовних екстрасистол під час руху риссю було виявлено у одного коня (2,7%). Результати дослідження підтверджують наявність аритмій у клінічно здорових коней української верхової породи. Тип аритмії достовірно залежить від періоду (спокій, навантаження, відновлення) активності тварини.*

Ключові слова: *аритмії, діагностика стану серця, портативний ЕКГ-монітор, реєстрація ЕКГ, коні*

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INFLUENCE OF PHOSPHOLIPID-CONTAINING ADDITIVES ON THE FUNCTIONAL CONDITION OF ORGANS AND SYSTEMS OF MICE

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Abstract. *Numerous studies in experiments on laboratory animals and in clinical practice have confirmed the positive effect of essential phospholipids on the structural organization of cell membranes, intracellular metabolism, mitochondrial function, antioxidant processes, which explains the active use of phospholipid-containing drugs in hepato- and enteropathologies, cardiovascular diseases, etc. The raw material of animal origin, in particular milk, which is a safe source of biologically active substances, has a structural similarity with phospholipids of mammalian tissues. Based on them, a biologically active additive (BAA) "FLP-MD" was created, which is characterized by membranotropic properties and reparative effect. The aim of this work was to investigate the biological effect of the liposomal form of BAA "FLP-MD" based on milk phospholipids on the body of clinically healthy laboratory mice.*

Determination of the toxicity of phospholipid-containing BAA "FLP-MD" was performed on mice of the CBA line. The duration of the experiment was 16 days. The results of the studies indicate a probable increase in body weight of mice of the CBA line after the 10th day of intragastric administration of 1% solution of liposomal form of BAA "FLP-MD".

As a result of the study of biochemical parameters of plasma / native blood, the absence of toxic effects of the liposomal form of BAA "FLP-MD" on the functional state of the body of mice was set. Morphological parameters of peripheral blood in these animals did not change significantly. According to the results of microscopic studies, no negative effect of the liposomal form of BAA "FLP-MD" on the structure microstructure of the mucous membrane of the gastrointestinal tract was found.

Therefore, the components of BAA "FLP-MD" do not adversely affect the functional state of organs and systems of laboratory mice and do not show a toxic effect, which allows it to be recommended for use in veterinary medicine as a means of rehabilitation therapy.

Keywords: *milk phospholipids, BAA "FLP-MD", laboratory mice of the CBA line, clinical, hematological and microscopic examinations, functional state of the organism*

Introduction

Now, the key role of structural and functional destabilization of cell membranes due to phospholipid deficiency, lipid peroxidation, and their hydrolysis in the pathogenesis of inflammatory, dystrophic and degenerative processes has been scientifically and experimentally proven (Solís-Calero et al., 2015; Gariani et al., 2017; Calitz et al., 2018). This indicates the feasibility of introducing reparative therapies in veterinary medicine and medicine, which include phospholipid-containing drugs, in particular, the most famous in the world – Essentiale® Forte N. Numerous studies in experiments on laboratory animals and when used in clinical practice have confirmed the positive effect of essential phospholipids on the structural organization of cell membranes, intracellular metabolism, the functional ability of mitochondria, antioxidant processes, and explains the active use of phospholipid-containing drugs in hepatopathology and enteropathology, cardiovascular diseases, the intoxication of the body, etc. (Bothra et al., 2018; Colombo et al., 2018; Yang et al., 2018; Zhang et al., 2018).

The structural similarities with phospholipids of mammalian tissues have raw materials of animal origin, in particular, obtained from milk, as well as a by-product of its processing – butterdish, which is a cheap and safe source of biologically active substances. Based on them, we created a biologically active additive (BAA) "FLP-MD", which is characterized by membrane-active properties and reparative effect of an action. Therefore, it is advisable to use reparative therapy in applied veterinary medicine, focused on the peculiarities of pathogenetic disorders, taking into account the natural potential and physiological ability of cells to self-restore lost functions, especially damaged intracellular structures – membrane systems. This approach, along with traditional therapy in the medical field, helps to reduce the economic damage from animal death, the occurrence of relapses and complications of relevant pathologies, as well as the solution of environmental, food. and biological safety problems.

Analysis of recent research and publications

Preventive medicine that studies the problems of common non-communicable diseases, based on the identification of risk factors for the disease. An urgent task is to assess the risk of developing a chronic process, the formation of which correlates with the duration of risk factors action and their intensity. A chronic pathological process is formed under the influence of risk factors for a long period. In this regard, to suspend it or achieve regression of the disease is possible only as a result of prolonged exposure to corrective factors while eliminating or attenuating the effects of negative factors. Inhibition of the progression of the pathological process and, especially, its reverse development is an extremely difficult task that requires the use of rehabilitation measures.

New rehabilitation technologies for rehabilitation treatment include endoecological rehabilitation aimed at improving the natural cavities and spaces formed by the serous and mucous membranes, endothelial structures, as well as the restoration of the extracellular and cellular structures (Gryshchenko et al., 2018). Currently, there are four levels of endoecological rehabilitation. Particularly noteworthy is the fourth level, which is aimed at stimulating cell regeneration and structural and functional restoration of cell formations: external and intracellular membranes, protoplasm, membrane receptors, nuclear

structures. The task of the fourth level of rehabilitation is solved by correcting the diet of animals with biologically active substances of membranotropic action, local action of membranotropic drugs (Gula & Margitich, 2009).

The criterion for assessing the effectiveness of endoecological rehabilitation of the fourth level can be methods that characterize the processes that occur in cell (biological) membranes. The membrane responds to external signals and induces the production of the corresponding metabolites by the cell; it is able to effectively rebuild in response to various factors. The physical state of the membrane is known to be determined by phospholipids, primarily phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine, which contain the bulk of the polyene fatty acids of the ω-6 and ω-3 structure (Gryshchenko, 2019; Sang-Chul & Xuemin, 2020).

In the process of rehabilitation therapy at the fourth level of endoecological interaction, there is a directed regulation of cellular lipid metabolism, which is carried out through the enzymatic block of desaturation of fatty acids, as well as due to the stabilization of vasodilator function in the synthesis of eicosanoids (Gryshchenko et al., 2019). Moreover, the intensity of lipid modification of cell membranes depends on the depth and nature of the initial metabolic disorders. The fourth level will be especially effective in rehabilitation for years, but the effect can be seen on several generations of the rehabilitated.

Most drugs from the arsenal of humane and veterinary medicine are artificially synthesized. Along with the quick effect of their use, the development of severe complications is possible, it is extremely dangerous in case of complex clinical situations (Gryshchenko et al., 2019; Starr et al., 2019). The likelihood of side effects requires a deliberate and careful prescription of such drugs in each case of the disease. Often the risk of negative effects of synthetic drugs on a living organism limits the possibility of their appointment due to existing contraindications. The vast majority of such drugs has cumulative properties and, if the recommended therapeutic and preventive doses are not followed, produces a toxic effect on the body.

The unqualified prescription of drugs, especially synthetic ones, often causes the development of a "drug" disease. According to statistical materials, the "drug" disease is the cause of death of about 100,000 people and the cause of the development of serious diseases in 2,200,000 people a year, unfortunately, no such studies have been conducted on the situation with animals. In many cases of "drug" disease, life-threatening side effects are noted: cardiac arrhythmia, renal failure, internal bleeding, and a critical decrease in blood pressure.

The use of natural, environmentally friendly raw materials with low cost to create BAA of therapeutic and prophylactic action should help reduce the negative impact of enteropathology on the health status of animals and the formation of their productive qualities (Tomchuk et al., 2019).

So, modern pharmaceutical production focuses on the search for new, efficient, environmentally friendly, and at the same time more cost-effective technologies; as a whole, it must solve an extremely important task – preserving human health and the environment. The components of dietary supplements can be various organic and mineral substances of natural origin, which, along with other properties of membranotropic action. These are phospholipids, essential fatty acids, amino acids, vitamins, etc. (Udut et al., 2013). The aim of the work was to investigate the

biological effect of the liposomal form of BAA "FLP-MD" based on milk phospholipids on the body of clinically healthy laboratory mice.

Materials and methods of research

All manipulations with rats were carried out in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for experimental and scientific purposes (Strasbourg, 1986), the Law of Ukraine "On the Protection of Animals from Cruelty" № 3447 from 02/21/2006, which was confirmed by the conclusion of the Bioethics Commission of the National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine.

The toxicity of the biologically active phospholipid-dietary supplement "FLP-MD" was determined in laboratory conditions on CBA mice by oral administration. The duration of the observation period is 16 days (Vlizlo et al., 2012).

These tests are widely used by toxicologists as a relatively quick, uncomplicated, low-cost, and statistically reliable method for assessing the negative effects of drugs on objects such as laboratory animals.

The studies were conducted on laboratory mice of the CBA line obtained from healthy individuals of the vivarium of the Institute of Oncology of the Academy of Medical Sciences of Ukraine. Used experimental mice that reached puberty at the beginning of the experiments. Healthy individuals were selected, which had approximately the same size and weight, had never before been used in other laboratory studies and manipulations. The weight of the mice was monitored throughout the experiment. So, at the beginning of the experiment, males weighed 19–20 g, females – 18–19 g.

To study the biological properties of the phospholipid-containing dietary supplement "FLP-MD" groups of experimental animals were composed of ten mice (five of each sex). Mice were kept in five individuals in a separate cage.

The drug was fed to mice every day for 16 days. Animals of the experimental group were intragastrically injected with a 1% solution of phospholipid-containing BAA "FLP-MD" in the liposomal form at a dose of 13.5 mg/kg once a day. BAA "FLP-MD" is the author's development (Melnychuk et al., 2009), which includes a complex of milk phospholipids (nipples) that have a fatty acid spectrum natural for mammalian cell membranes, a mixture of unsaturated fatty acids (oleic, linoleic, linolenic), obtained with linseed oil, and antioxidants (vitamins A and E). The control group included intact animals injected *per os* with an equivalent volume of distilled water. After administration of the test substance, the mice had free access to food and water. Food intake was monitored by both test and control mice.

We observed changes in the general condition of the animals, the presence of symptoms of intoxication. Every 24 hours of the experiment, the mice were weighed and the amount of feed consumed was monitored. After the experiment, the mice were decapitated under ether anesthesia, blood samples were taken for morphological and biochemical studies, stomach and intestine samples for microscopic studies. The blood was stabilized with heparin. Morphological (number of erythrocytes, leukocytes, platelets, leukogram) and physical (hematocrit, erythrocyte sedimentation rate, ESR) parameters of blood were calculated and measured by the method of Coulter on a veterinary hematology analyzer "Mindray BC 2800 Vet" (China)

(Vlizlo et al., 2012). Hemoglobin content was determined in whole blood using the Analyzer Micro CC-20 Plus Auto HTI (USA) according to the instructions of the manufacturer. Biochemical indicators were determined by biochemical GBG Stat Fax 1904 Plus (Awareness Technology, Inc., Florida, USA) using chemicals purchased from DAC-SPECTROMED S.R.L. (Republic of Moldova). This device allowed to determine routinely the content of total protein, glucose; activity of enzymes e.g. aspartate transaminase (AST, EC 2.6.1.1), alanine transaminase (ALT, EC 2.6.1.2), alkaline phosphatase (ALP, EC 3.1.3.1) and lactate dehydrogenase (LDH, EC 1.1.1.27). Microscopic samples were stained with hematoxylin-eosin, blood smears according to Romanovsky-Giemsa.

Statistical data analysis was performed using software Statistica 6.0. Data confidence was estimated using Student t-criteria. Data were assumed as significant at $P < 0.05$.

Results of the research and their discussion

Throughout the experiment, the behavior of animals in the experimental group did not differ from that in mice of the control group. When assessing the appearance (condition of the skin, coat, mucous membranes) in mice of the experimental group, no differences were revealed compared with animals of the control group. After administration of the BAA "FLP-MD", no symptoms of intoxication (tremors, seizures, paresis, and paralysis) were observed in animals of the experimental group.

During visual observation in experimental animals, the frequency of urine output and defecation, as well as the color of urine and feces did not differ from those in the control group.

The dynamics of changes in the body weight of CBA mice of various groups are given in Table. 1. According to the table, when feeding a phospholipid-containing

BAA "FLP-MD" to mice for 16 days, a significant increase in the bodyweight of mice was observed after 10 days of experiment in both males and females.

The results of the study of morphological, biochemical, and physical parameters of the peripheral blood of mice treated with phospholipid-positive BAA "FLP-MD" for 16 days are presented in Table. 2.

According to the results presented in mice of the experimental group, the number of erythrocytes and the ESR decrease under the influence of phospholipids of the dietary supplement, which is better expressed in females (correspondingly from $(7.22 \pm 0.25) \times 10^{12}$/L to $(6.04 \pm 0.25) \times 10^{12}$/L and from (1.6 ± 0.24) mm/h to (0.8 ± 0.2) mm/h, $P < 0.05$). All other indicators of animals treated with BAA "FLP-MD" did not change significantly.

As a result of biochemical studies, which are presented in Table 3, when mice were fed phospholipids of milk in the form of liposomal BAA "FLP-MD" for 16 days, no significant changes in blood plasma parameters were found, with the exception of alanine transaminase (ALT, EC 2.6.1.2) in females.

The above allows us to conclude that there is no toxic effect of the components of the dietary supplement on the functional condition of the body.

The results of microscopic studies of the stomach and intestines. On the preparation of the esophagus in females of the experimental group, the transition of the stratified squamous epithelium of the esophagus into the glandular epithelium of the cardiac part of the stomach and the absence of cytological or structural

1. The average change in bodyweight of mice (g) over the experimental period (M ± m, n = 5 in each group)

Group of animals		Bodyweight (g)				
		2 day	5 day	8 day	10 day	16 day
Experimental Group	females	17.7 ± 0.3	18.4 ± 0.1	18.6 ± 0.3	18.8 ± 0.3*	19.0 ± 0.3*
	males	19.5 ± 0.4	20.5 ± 0.6	21.1 ± 0.6	22.1 ± 0.4*	22.9 ± 0.3*
Control Group	females	18.1 ± 0.2	18.6 ± 0.3	18.7 ± 0.2	18.9 ± 0.2	19.7 ± 0.6
	males	21.0 ± 0.3	22.0 ± 0.6	20.9 ± 0.7	20.9 ± 1.0	21.1 ± 1.3

Note: * $P < 0.05$, compared with the initial data on the 2nd day of the experiment in the context of the control groups.

changes in these organs are noted. In the esophagus, strata of the stratified epithelium are surrounded by keratin masses with foci of mineralization (dystrophic liming). Dystrophic liming indicates the antiquity of the mineralization process and is not a pathology. No structural changes were detected in the epithelial cells of the esophagus glands and the cardiac glands of the stomach. The muscular membrane with an active response of smooth muscle cells, the serous membrane is thickened with an active reaction of fibroblasts. At the intersection of the esophagus into the

2. Blood morphological, biochemical and physical parameters in experimental mice (M ± m, n = 5 in each group)

Indicator		Males		Females	
		experimental group	control group	experimental group	control group
Erythrocytes, x 10^{12}/L		6.01 ± 0.25*	7.22 ± 0.25	6.97 ± 0.51	7.60 ± 0.66
Hemoglobin, g/L		112.0 ± 1.7	116.8 ± 2.7	116.3 ± 3.03	115.3 ± 1.8
Hematocrit, conv. units		0.46 ± 0.03	0.45 ± 0.01	0.47 ± 0.01	0.43 ± 0.02
Platelets, x 10^9/L		548.67 ± 145.60	515.20± 41.20	611.25 ± 26.56	607.25 ± 39.02
Leukocytes, x 10^9/L		6.78 ± 0.56	6.80 ± 0.44	5.97 ± 0.35	5.40 ± 0.77
Stab leukocytes	10^9/L	0.021 ± 0.014	0.040 ± 0.023	0.051 ± 0.021	0.056 ± 0.021
	%	0.30 ± 0.20	0.20 ± 0.12	0.83 ± 0.33	1.00 ± 0.36
Segmental leukocytes	10^9/L	1.08 ± 0.06	1.06 ± 0.28	1.07 ± 0.25	1.14 ± 0.19
	%	16.60 ± 1.96	15.00 ± 3.38	17.67 ± 3.77	21.29 ± 2.93
Eosinophiles	10^9/L	0.17 ± 0.05	0.22 ± 0.08	0.15 ± 0.03	0.13 ± 0.02
	%	2.50 ± 0.63	3.30 ± 1.35	2.58 ± 0.45	2.71 ± 0.56
Monocytes	10^9/L	0.22 ± 0.05	0.39 ± 0.14	0.28 ± 0.03	0.33 ± 0.09
	%	3.20 ± 0.66	5.50 ± 1.75	4.75 ± 0.64	6.21 ± 1.60
Lymphocytes	10^9/L	5.30 ± 0.56	5.13 ± 0.20	4.44 ± 0.38	3.73 ± 0.68
	%	77.60 ± 2.14	76.00 ± 2.76	74.50 ± 4.61	68.36 ± 4.58
ESR, mm/hour		0.80 ± 0.10*	1.60 ± 0.14	1.33 ± 0.17	1.51 ± 0.29

Note: * $P < 0.05$, the difference is significant compared with the control.

3. Blood plasma biochemical parameters in mice of different groups (M ± m, n = 5 in each group)

Indicator	Males		Females	
	experimental group	control group	experimental group	control group
Alanine transaminase, IU	126.3 ± 6.1	139.3 ± 1.9	97.0 ± 1.6*	83.3 ± 1.1
Aspartate transaminase, IU	565.6 ± 30.7	612.3 ± 57.9	537.0 ± 50.1	527.6 ± 71.6
Alkaline phosphatase, IU	864.0 ± 16.2*	755.0 ± 31.3	792.3 ± 45.3	969.0 ± 73.9
Lactate dehydrogenase, IU	2496.3 ± 203.2	2996.3 ± 79.9	10199.0 ± 102.7	10802.0 ± 488.1
Glucose, mM	20.7 ± 1.2	19.4 ± 0.8	17.8 ± 0.6	18.21 ± 1.5
Total protein, g/L	39.3 ± 1.9	37.3 ± 0.6	31.0 ± 0.4	31.0 ± 1.2

Note: * $P < 0.05$, the difference is significant compared with the control.

stomach, there are massive round-cell infiltrates (inflammatory), stasis (plethora) of blood vessels. The mineralization of keratin masses in the esophagus and at the same time an active reaction of the basal cells of the esophagus are noted.

The mucous membrane of the stomach was rejected into the lumen with stick necrosis fragments of the glandular epithelium and the storage of individual Brunner glands, which are represented by a cylindrical epithelium. The rejection of the gastric mucosa extends to the limit of its plate. The last thinned, homogeneous, poor cells with signs of mineralization. The muscular membrane is thinned with signs of intermuscular edema; smooth muscle cell response and lymphocyte infiltration. Some preparations have no structural changes. All layers of the structure are clearly recognizable: lobes, fossa of the mucosa, and own glands. The submucosa of the mucosa membrane is thinned, the muscle and serous membranes are thickened.

The small intestine corresponds to the structure of the duodenum. The structure of the mucous membrane is preserved, despite the rejection of the mucous layers in the intestinal lumen and necrosis of a part of the villi. The submucosa and serous membrane are absent. In the stroma of the intestinal villi, lymphocytic infiltration is expressed. The structure of the small intestine is not broken in all layers. The villi of the mucous membrane are large with a moderate endothelial reaction. The cavernous expansion of the blood vessels of the submucosal layer is determined. Hypertrophied muscle plate (middle section). The serous membrane is absent.

In males, when studying the preparations in the transitional zone of the esophagus and the cardiac part of the stomach, the hyperkeratinization between the cells of the stratified squamous epithelium and the subepithelial layer with foci of mineralization (dystrophic liming) is noted. Massive foci of hemorrhage (intramuscular). The active reaction of nuclear forms of fat cells in the upper section. Fragmentation and decay of connective tissue fibers, which borders the squamous epithelium.

Transitional zone of the esophagus into the stomach: on the verge of a multilayer squamous epithelium of the esophagus and glandular epithelium of the cardiac section of the stomach, round-cell infiltrates were found. Dystrophic liming in the intermediate tis-

sue of the esophagus. The structure of the stomach corresponds to the structure of the normal mucous membrane with pyloric glands and muscle membrane.

Small intestine: pockets crypts are clearly distinguished, villi are flattened, sent by a single-layer cylindrical epithelium, without visible pathological changes, mainly edged in the absence of other cells that make up the intestinal villus (goblet-like mucus-producing and endocrine-hormone-producing). Edema of the intermediate tissue of the Brunner glands with microcenters of inflammatory round-cell infiltrates was revealed.

When studying the transition of the esophagus to the cardiac stomach in males and females of the control group, the pattern of changes corresponds to the characteristic changes for the experimental group described above, with the difference that a more pronounced inflammatory reaction is noted in the underlying connective tissue stroma of the esophagus. It turns dystrophic liming subepithelial tissue of the esophagus. The cardiac glands of the esophagus (Brunner) are not structurally altered; there is intra-plasmic retardation of mucus with infiltration of the submucosal layer of lymphocyte.

In the plate of the mucous membrane of the esophagus, the structure is not broken, it contains all types of cells with a predominance of mucus-producing. In structure, it does not differ from the mucous membrane, characteristic for normal. In the small intestine, high villi were detected with a pronounced reaction of lymphocytes and plasma cells in their plate of the mucous membrane.

Conclusions and Prospects

During the feeding mice of the CBA line with the liposomal form of BAA "FLP-MD" based on milk phospholipids (within 16 days), a significant increase in body weight was observed after the 10th day of the experiment in both males and females. The amount of feed consumed during the entire period of the experiment in the control and experimental groups do not differ.

The obtained biochemical blood parameters indicate the absence of toxic effects of the liposomal form of BAA "FLP-MD" based on milk phospholipids on the functional condition of the body.

The morphological parameters of the peripheral blood in experimental animals receiving the liposomal form of BAA "FLP-MD" do not change significantly, with the exception of a decrease in the number of red blood cells and the ESR in animals of the experimental group.

According to the results of microscopic studies, there was no negative effect of the liposomal form of BAA "FLP-MD" based on milk phospholipids on the microstructures of the mucous membrane of the organs of the gastrointestinal tract. The normal structure of this section of the digestive tract is distinguished only by pronounced inflammatory infiltration (squamous) in the submucosal layer. Hyperplasia of the lymphoid elements of Peyer's plaques is noted. The epithelial cells of the Brunner glands are filled with mucus (delayed withdrawal), like the glands of the cardiac section of the stomach.

Therefore, the BAA "FLP-MD" can be recommended for use in veterinary medicine as a means of rehabilitation therapy.

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Анотація. *Численними дослідженнями в експериментах на лабораторних тваринах і під час використання в клінічній практиці підтверджено позитивний вплив есенційних фосфоліпідів на структурну організацію клітинних мембран, внутрішньоклітинний метаболізм, функціональну здатність мітохондрій, антиоксидантні процеси, що пояснює активне застосування фосфоліпідовмісних препаратів за гепато- та ентеропатологій, серцево-судинних захворювань тощо. Структурну подібність із фосфоліпідами тканин ссавців має сировина тваринного походження, зокрема, молока, яка є безпечним джерелом біологічно активних речовин. На їхній основі створено біологічно активну добавку (БАД) «FLP-MD», для якої характерні мембранотропні властивості та репаративний ефект дії. Метою роботи було дослідити біологічний вплив ліпосомальної форми БАД «FLP-MD» на основі фосфоліпідів молока на організм клінічно здорових лабораторних мишей.*

Визначення токсичності фосфоліпідовмісної БАД «FLP-MD» проводили на мишах лінії СВА. Тривалість досліду становила 16 діб. Результати досліджень свідчать про вірогідне збільшення маси тіла мишей лінії СВА після 10-ої доби інтрагастрального введення 1% розчину ліпосомальної форми БАД «FLP-MD».

У результаті дослідження біохімічних показників плазми/нативної крові встановлено відсутність токсичного впливу ліпосомальної форми БАД «FLP-MD» на функціональний стан організму мишей. Морфологічні показники периферичної крові цих тварин не зазнавали істотних змін. За результатами мікроскопічних досліджень не виявлено негативного впливу ліпосомальної форми БАД «FLP-MD» на мікроструктуру слизової оболонки органів шлунково-кишкового тракту.

Отже, складові БАД «FLP-MD» не чинять негативного впливу на функціональний стан органів і систем організму лабораторних мишей та не виявляють токсичного ефекту дії, що дозволяє її рекомендувати для застосування у ветеринарії в якості засобу відновлювальної терапії.

Ключові слова: *фосфоліпіди молока, БАД «FLP-MD», лабораторні миші лінії СВА, клінічні, гематологічні та мікроскопічні дослідження, функціональний стан організму*

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CELLULAR COMPOSITION OF LYMPHOID TISSUE OF PEYER'S PATCH OF DUODENUM IN DUCKS

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Abstract. *The aim of our research was to study the cell composition of the lymphoid tissue of the duodenal Peyer's patch of the ducks in age-related aspects. Among the cells of the lymphoid tissue of the duodenal Peyer's patch are immunoblasts, lymphocytes, proplasmocytes, and plasma cells, monocytes and macrophages, epithelial cells, reticular cells, erythrocytes, granulocytes, fibroblasts, and M-cells. The content of populations of these cells is not the same. Lymphocytes in the preparations-imprints are found the most. They are represented by small, medium, and large forms. Small lymphocytes were found in the largest number, and large ones – in the smallest. The content of small lymphocytes decreases unevenly with the age of ducks, and medium and large lymphocytes – increases unevenly. The content of immunoblasts in the lymphoid tissue of the duodenal Peyer's patch of ducks is less than that of lymphocytes. This index decreases unevenly from the 1st to the 420 days of age of birds. Proplasmocytes and plasmocytes are found in the lymphoid tissue of the duodenal Peyer's patch of ducks in small numbers from the 15th day of age. Their content increases significantly with the increasing of the birds' age. The content of macrophages and monocytes in the lymphoid tissue of the duodenal Peyer's patch of ducks is insignificant. Uneven increase of this indicator is registered from the 1st to the 420 days of age of birds. The content of reticular cells cannot be calculated because they are covered by lymphoid cells, and fibroblasts, erythrocytes, granulocytes, and M-cells that occur in the imprint preparations in small quantities cannot be statistically processed.*

Keywords: *ducks, duodenum, Peyer's patch, lymphoid tissue, immunoblasts, lymphocytes, proplasmocytes, plasmocytes, monocytes, macrophages*

Introduction

It is well known that the organs of hemo- and lymphopoiesis include central (primary) and peripheral (secondary) organs, as well as focal clusters of lymphocytes scattered throughout the body, including lymphocytes circulating in the bloodstream and lymph (Day & Schultz, 2014; Kaushansky et al., 2016). A special place in this multicomponent tissue organization is given to the so-

called lymphoid tissue associated with mucous membranes (mucous associated lymphoid tissue – MALT). In birds, MALT is formed by lymphoid cells, which are localized in their own plate and submucosal basis of the mucous membrane of the gastrointestinal tract (GALT) and respiratory tract (BALT) (Day & Schultz, 2014; Kaushansky et al., 2016). These structures form the first protective barrier against antigens that enter the body with food and air. Interest in GALT in birds is currently growing, due to the fact that the vast majority of antigens enter the body of these animals through the digestive organs (Forchielli & Walker, 2005). As a result, about 70% of the lymphoid tissue, which forms the parenchyma of peripheral organs of immunogenesis, including GALT structures that comprise Peyer's patches, is localized in their walls (Neutra et al., 2001).

All functions of lymphoid tissue provide its cellular elements from polypotent stem cells to effector cells (lymphocytes, plasma cells, macrophages), which are in the processes of proliferation, differentiation, migration, cooperation, and apoptosis (Regoli et al., 1994; Trufakin et al., 2005). The optimal balance of immunocompetent cells provides its main function – immunity. We did not find information on the cellular composition of the lymphoid tissue of the duodenal Peyer's patch of the ducks in the special literature. Therefore, the study of this issue is relevant.

Materials and methods of researches

Intestines were obtained from the Blahovarsky cross broiler ducks of 1, 5, 10, 15, 20, 25, 30, 60, 90, 120, 150, 180, 210, 240 days old (5 ducks per group). All interventions and slaughter of the birds were performed in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Council of Europe, 1987) and the Decree of the First National Congress on Bioethics (Reznikov, 2003).

Cytological examinations were performed on imprint preparations stained with commercial stains LeikoDiff 200 (Erba Lachema, Czech Republic) and with Pappenheim's stains Hemacolor (Merck, Germany) (Silverman & Frable, 1990; Storc et al., 2005). The slides were examined with a bright-field microscope "Olympus" (Japan). In imprint preparations, cells were differentiated and their number was counted in 5 fields of view of the microscope (in one preparation). 50–70 cells were counted in one field of the microscope (Avtandilov, 1990).

The arithmetic mean was determined statistically with the error of the arithmetic mean (SD). Statistical analysis was performed by StatSoft Statistica 13.1 (1998).

Results of the research and their discussion

Immunoblasts, lymphocytes, proplasmocytes and plasmocytes, monocytes and macrophages, epithelial cells, reticular cells, erythrocytes, granulocytes, and M-cells were found in imprint preparations of the lymphoid tissue of the Peyer's patch in ducks by age-related studies. The presence of these cells in the immune formations of the intestines in birds has been reported by other researchers (Burns & Maxwell, 1986; Havrylin et al., 2014; Barsukova et al., 2015; Qu, 2018). The content of these cell populations is not the same (Tables 1, 2). The content of reticular

cells cannot be calculated because they are covered by lymphoid cells, and fibroblasts, erythrocytes, granulocytes, and M-cells occur in the imprint preparations in small quantities that cannot be statistically processed.

Lymphocytes are mostly found among the cells (Table 1). They have a structure similar to that in mammals and other birds. The lymphocytes on the imprint preparations have a predominantly round shape and a large basophilic nucleus, which occupies almost the entire volume of the cell (Fig. 1). The shape of the nuclei is different. They can be rounded and slightly elongated. The contours of the nuclei are uneven. A lot of heterochromatin is attached to the inner membrane of their cell wall. The latter, in the form of grains and lumps, is also freely present throughout the nucleoplasm. The nucleolus in the nucleus is difficult to differentiate due to the high content of heterochromatin. The cytoplasmic volume of lymphocytes is insignificant. It surrounds the nucleus in the form of a thin weakly basophilic strip.

The lymphocytes content in ducks of the studied age groups decreases unevenly, which, in our opinion, is due to their differentiation into effector cells. Thus, in one-day-old this indicator is 65.32 ± 0.39%, and in 420-day-old – 55.97 ± 0.32%. The content of lymphocytes decreases most intensively in ducks in age from 10 to 15 days (by 2.95%).

Among lymphocytes are small, medium, and large. Their content is also different (Table 2). Small lymphocytes are found most in ducks of all ages. Their diameter is up to 7 microns. Small lymphocytes have a large nucleus. Due to the high heterochromatin content, it is intensely stained. The nucleus is surrounded by a narrow strip of cytoplasm. The cytoplasm of small lymphocytes has a form of a crescent-shaped strip,

1. The content of cells in the Peyer's patch of the duodenum in ducks (M ± m,%)

Age, days	Immunoblasts	Lymphocytes	Proplasmocytes and plasmocytes	Monocytes and macrophages
1	32.64 ± 0.36	65.32 ± 0.39	–	2.04 ± 0.09
5	32.53 ± 0.24	65.09 ± 0.25	–	2.38 ± 0.09
10	32.52 ± 0.09	64.76 ± 0.21	–	2.73 ± 0.17
15	31.49 ± 0.26	62.86 ± 0.24	2.52 ± 0.11	3.14 ± 0.19
20	31.63 ± 0.35	61.91± 0.11	2.77 ± 0.16	3.69 ± 0.18
25	30.28 ± 0.17	61.53 ± 0.17	3.63 ± 0.10	4.56 ± 0.23
30	28.04 ± 0.19	60.88 ± 0.19	6.91 ± 0.28*	4.17 ± 0.07
60	26.51 ± 0.49	60.89 ± 1.42	9.21 ± 0.78	3.39 ± 0.25
90	25.59 ± 0.76	60.94 ± 0.75	10.41 ± 0.61	3.06 ± 0.59
120	25.94 ± 0.19	60.25 ± 0.26	9.19 ± 0.37	4.62 ± 0.16
150	24.06 ± 0.29	59.65 ± 0.80	9.66 ± 0.44	6.63 ± 0.26*
180	24.72 ± 0.37	58.94 ± 0.19	9.54 ± 0.26	6.79 ± 0.25
210	25.86 ± 0.26	58.23 ± 0.27	9.85 ± 0.23	6.07 ± 0.17
240	24.76 ± 0.16	56.58 ± 0.26	11.24 ± 0.23	7.42 ± 0.13
330	24.15 ± 0.11	56.99 ± 0.27	10.83 ± 0.12	8.02 ± 0.24
420	24.48 ± 0.08	55.97 ± 0.32	11.13 ± 0.35	8.42 ± 0.28

Note: * $P < 0.05$ compared to the previous age group.

which does not completely surround the nucleus (Fig. 1). The content of these lymphocytes decreases unevenly from age of one-day (52.27 ± 0.51%) to 420-day (48.65 ± 0.49%). The content of small lymphocytes decreases most intensively in ducks from 60 to 90 days of age (by 9.71 %).

Medium lymphocytes are larger (d = 7–10 μm) than small ones. They have a larger volume of cytoplasm and nucleus. In their nucleus, the heterochromatin content is slightly lower, as a result of which they have a less intense colour (Fig. 1). The content of medium lymphocytes in the lymphoid tissue of the duodenal Peyer's patch in ducks of the studied age groups decreases unevenly (Table 2). Thus, in 1-day-old birds, this indicator is 40.10 ± 0.82%, while in 420-day-old – 36.96 ± 0.43%. The content of medium lymphocytes increases more intensively in ducks aged from 30 to 60 days (by 7.68%).

The content of large lymphocytes on the imprint preparations is the lowest. The volume of their cytoplasm and nucleus is the largest (Fig. 1). The nucleus is weakly basophilic, contains small lumps of heterochromatin. The cytoplasm is manifested in the form of a thin weakly basophilic strip. The content of large lymphocytes in ducks of the studied age groups increases unevenly (Table 2). In 1-day-old birds their content is 7.63 ± 0.67%, and in 420-day-old birds – 14.39 ± 0.68%. The most intensive increase in the content of large lymphocytes (by 52.06%) is observed in ducks from 30 to 60 days of age.

Immunoblasts – cells of the fifth class of lymphocytopoiesis, precursors of effector cells of lymphocytes (Davison et al., 2008). Their size is larger than small and medium lymphocytes. Immunoblasts have a shape from round to slightly elongated. The volume of their cytoplasm is much larger than that of lymphocytes (Fig. 2).

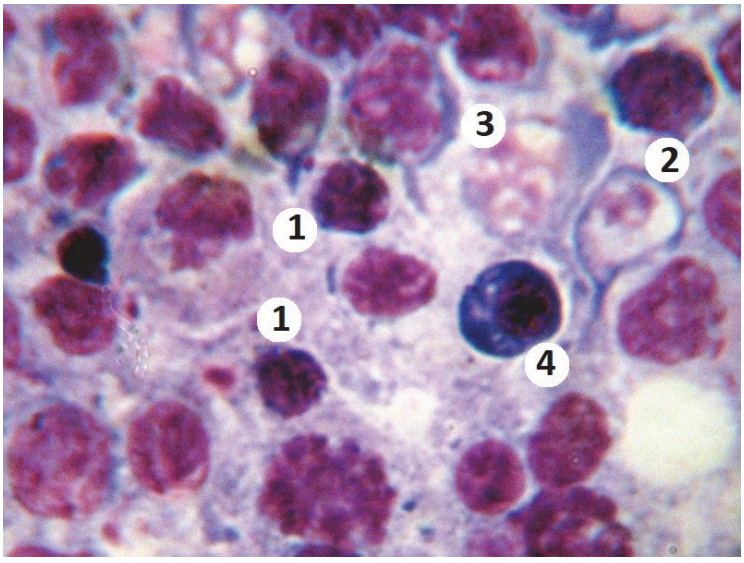

Fig. 1. Cells of the duodenal Peyer's patch of in duck aged 150 days:
1 – small lymphocyte; 2 – medium lymphocyte; 3 – large lymphocyte; 4 – plasma cell. Imprint preparation. Wright staining, × 1000

2. The content of different groups of lymphocytes in the Peyer's patch of the duodenum in ducks (M ± m,%)

Age, days	Lymphocytes		
	Small	Medium	Large
1	52.26 ± 0.51	40.10 ± 0.82	7.63 ± 0.67
5	51.77 ± 0.48	40.04 ± 1.03	8.19 ± 0.72
10	51.09 ± 0.44	40.59 ± 0.79	8.32 ± 0.41
15	53.48 ± 0.22	39.07 ± 0.21	7.46 ± 0.29
20	53.69 ± 0.15	38.91 ± 0.16	7.39 ± 0.31
25	54.23 ± 0.94	37.87 ± 0.67	7.89 ± 0.41
30	51.54 ± 0.89	37.58 ± 0.69	9.02 ± 0.71
60	49.75 ± 0.92	35.12 ± 1.58	15.13 ± 1.05
90	44.92 ± 1.27	38.53 ± 1.41	16.56 ± 1.18
120	46.63 ± 0.39	39.61 ± 0.73	14.76 ± 0.57
150	45.75 ± 1.11	38.41 ± 0.78	15.84 ± 0.77
180	46.12 ± 0.27	38.21 ± 0.17	15.67 ± 0.21
210	47.56 ± 0.55	36.14 ± 0.28	16.29 ± 0.78
240	47.74 ± 0.38	36.92 ± 0.35	15.34 ± 0.15
330	47.82± 0.36	36.42 ± 0.19	15.76 ± 0.44
420	48.65 ± 0.49	36.96 ± 0.43	14.38 ± 0.68

Note: * $P < 0.05$ compared to the previous age group.

It, in the form of a narrow basophilic strip of unequal thickness, completely or partially delimits the nucleus. It reveals a small number of large round mitochondria with a light matrix and weakly expressed crystals, elements (tubules and cisterns) of the endoplasmic reticulum, elements of the Golgi complex, lysosomes, polyribosomes. The nucleus is light spherical, contains mainly two nucleoli, chromatin is evenly distributed in the nucleoplasm. Part of the heterochromatin is fixed near the inner membrane of the nuclear envelope.

The content of immunoblasts in the lymphoid tissue of the duodenal Peyer's patch in ducks is less than that of lymphocytes (Table 1). In birds of all studied age groups, the content of immunoblasts decreases unevenly from one day (32.64 ± 0.36%) to 420 days of age (24.48 ± 0.08%). The content of immunoblasts decreases most intensively in ducks from 25 to 30 days of age (by 7.4%).

Plasmocytes – effector cells of B-lymphocytes, and proplasmocytes – precursors of plasma cells. Proplasmocytes are small, irregularly shaped cells. Their nucleus with jagged edges has an irregular shape. The nucleus contains one or two nucleoli, in the nucleoplasm, there is a lot of heterochromatin that, fixing to the inner membrane of the nucleolemma, forms triangular and trapezoidal shapes. The cytoplasm is basophilic and larger than that of lymphocytes. Elements of the granular endoplasmic reticulum and mitochondria are localized in the cytoplasm.

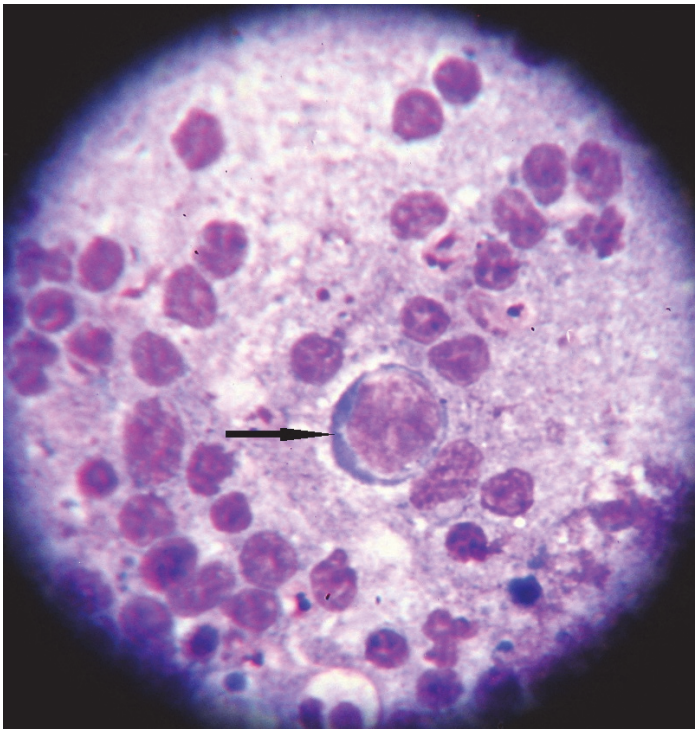

Fig. 2. Immunoblast (arrow) of the duodenal Peyer's patch in a 90-day-old duck. Imprint preparation. Wright staining, × 1000

Plasmocytes – cells of small size, round, or oval shape with an eccentrically located nucleus (Fig. 1). In the latter, well-condensed heterochromatin is pronounced, forming a characteristic pattern in the form of wheel spokes or a clock face. In the cytoplasm near the nucleus, a visible area of enlightenment is the Golgi complex. The volume of the cytoplasm exceeds the volume of the nucleus and it is almost completely filled with the granular endoplasmic reticulum, the tubules of which are significantly expanded. The latter are located mainly concentrically around the nucleus. A few large mitochondria of oval and round shape with a light matrix, ribosomes, and their clusters are also visible in the cytoplasm.

Plasmocytes and proplasmocytes in the lymphoid tissue of the duodenal Peyer's patch in ducks are detected from the 15th days of age in small quantities (2.52 ± 0.11%) (Table 1). With the increase in the age of birds, the content of these cells increases significantly (by 341.66%) and in 420-day-old ducks is 11.13 ± 0.35%. In ducks in age from 10 to 15 days, the most intense, almost double (by 90.36%) increase in the content of proplasmocytes and plasma cells is registered.

Monocytes are precursors of macrophages. They are large cells with a bean-shaped or horseshoe-shaped nucleus. In the nucleus, the lumps of heterochromatin are evenly distributed throughout the nucleoplasm. There are rounded mitochondria with a light matrix, lysosomes, and endoplasmic reticulum in the cytoplasm.

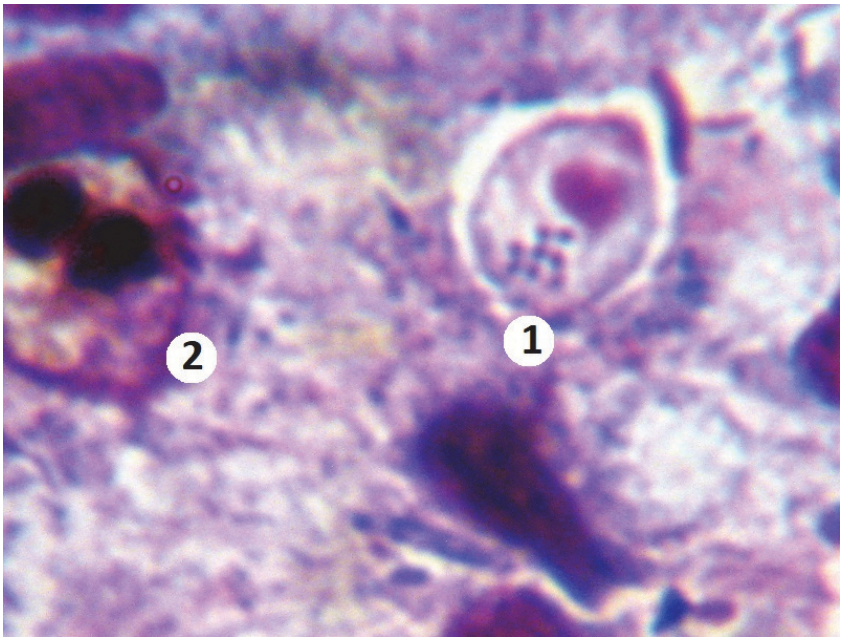

Fig. 3. Cells of the duodenal Peyer's patch in a 210-day-old duck. Imprint preparation: 1 – macrophage; 2 – pseudoeosinophil. Wright staining, × 1000

Macrophages are large, round, or oval-shaped cells with jagged edges (Fig. 3). The nucleus of macrophages is small, it has a lot of heterochromatin, which is fixed to the inner membrane of the nuclear envelope and sprayed into the nucleoplasm. The cytoplasm has a significant volume and forms protrusions of various sizes and shapes. It has many large round lysosomes, small phagosomes, slightly fewer mitochondria, and tubules of the endoplasmic reticulum.

The content of macrophages and monocytes in the lymphoid tissue of the duodenal Peyer's patch in ducks of the studied age groups is also insignificant. This figure increases unevenly from 2.04 ± 0.09% in 1-day-old birds to 8.42 ± 0.28% in 420-day-old birds. The most intensive increase in the number of monocytes and macrophages in lymphoid tissue of the duodenal Peyer's patch of ducks is registered in age from 90 to 120 days (by 50.98%).

Conclusions

1. Among the cells of the lymphoid tissue of the duodenal Peyer's patch are immunoblasts, lymphocytes, proplasmocytes and plasma cells, monocytes, and macrophages. The content of populations of these cells is not the same.
2. Lymphocytes in the imprint preparations are found the most. They are represented by small, medium, and large forms. Small lymphocytes were found in the largest number, and large – in the smallest. The total content of lymphocytes decreases by 14.31% with the age of ducks. The content of small and medium lymphocytes decreases unequally by 6.91 and 7.83%, respectively, and large – unevenly increases by 88.47%.
3. The content of immunoblasts in the lymphoid tissue of the duodenal Peyer's patch is unequally reduced by 25.0% with the age of ducks.

4. Proplasmocytes and plasmocytes in the lymphoid tissue of the duodenal Peyer's patch in ducks are detected from the 15th days of age. Their content increases by more than 4 times (by 341.67%) with the age of birds.
5. The content of macrophages and monocytes in the lymphoid tissue of the duodenal Peyer's patch of ducks unequally increases more than 4 times (by 312.75%) with the age.

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Анотація. *Метою нашого дослідження було вивчити клітинний склад лімфоїдної тканини плямки Пейєра дванадцятипалої кишки качок у віковому аспекті. Серед клітин лімфоїдної тканини плямки Пейєра дванадцятипалої кишки виявляються імунобласти, лімфоцити, проплазмоцити та плазматичні клітини, моноцити та макрофаги, епітеліальні клітини, ретикулярні клітини, еритроцити, гранулоцити, фібробласти та М-клітини. Вміст популяцій цих клітин неоднаковий. Лімфоцитів у препаратах-відбитках виявляється найбільше. Вони представлені малими, середніми і великими формами. Малих лімфоцитів реєструється найбільше, а великих – найменше. Вміст малих і середніх лімфоцитів із віком качок нерівномірно зменшується, а великих лімфоцитів – нерівномірно збільшується. Вміст імунобластів у лімфоїдній тканині плямки Пейєра дванадцятипалої кишки качок менший такого лімфоцитів. Цей показник нерівномірно зменшується від першої до 420 доби життя птиці. Проплазмоцити і плазмоцити в лімфоїдній тканині плямки Пейєра дванадцятипалої кишки виявляються в невеликій кількості з 15-добового віку качок. Їхній вміст значно збільшується зі збільшенням віку птиці. Вміст макрофагів та моноцитів у лімфоїдній тканині плямки Пейєра дванадцятипалої кишки качок незначний. Від першої до 420 доби життя птиці реєструється нерівномірне збільшення цього показника. Водночас, вміст ретикулярних клітин підрахувати неможливо, оскільки вони прикриті лімфоїдними клітинами, а фібробласти, еритроцити, гранулоцити і М-клітини трапляються в препаратах-відбитках у незначній кількості, яка не піддається статистичній обробці.*

Ключові слова: *качки, дванадцятипала кишка, плямка Пейєра, лімфоїдна тканина, імунобласти, лімфоцити, проплазмоцити, плазмоцити, моноцити, макрофаги*

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MODELLING THE SPREAD OF RABIES IN UKRAINE

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Abstract. *Due to the concern for animal and human health, efforts to develop mathematical and computational models for predicting the spatial and temporal dynamics of infectious diseases, including rabies, are constantly increasing. Therefore, our goal was to model the dynamics of the spread of rabies by foxes, dogs, and cats on the territory of Vinnytsia Oblast in the period from 2012 to 2018. To study and analyse the dynamic spread of rabies in time and space, an information-analytical technology was developed, which is based on retrospective data from registered cases of rabies. The rabies transmission parameter was determined using official epizootological data from the regional oblast veterinary laboratory and the regional department of the State Service of Ukraine On Food Safety and Consumer Protection. It was found that rabies spread the most among dogs and cats at a distance of up to 50 km in the winter (26.45%), from 50 to 100 km – in the autumn (40.33%), over 100 km – in the summer (49.84%). In general, domestic carnivores were sources of rabies within a radius of 50 km in only 16.98% of cases, and within a radius of 100 km – in 55.95% of cases. Foxes were sources of rabies within a radius of 50 km in only 24.33% of cases and within a radius of 100 km in 58.54% of cases. At the same time, the highest spread of rabies among the foxes at a distance of up to 50 km was observed in the spring (31.01%), from 50 to 100 km – in the winter (43.67%), and over 100 km – in the summer (48.38%). The new data will be useful in planning and carrying out preventive anti-rabies measures. In the future, it is planned to conduct the modelling in terms of all regions and create forecasts for the spread of rabies on the territory of Ukraine.*

Keywords: *animal rabies, rabies distribution modelling, foxes, domestic carnivores, information-analytical technology*

Introduction

Rabies infections are the cause of over 99 % of all human rabies deaths, and therefore pose a serious threat to public health worldwide. Rabies viruses are transmitted to a wide range of globally distributed mammals, but only some species are able to be a reservoir. The twentieth century was significant

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in the eradication of rabies due to effective attempts to eradicate rabies in the wildlife (Robardet et al., 2019) as well as by eliminating rabies from a number of European countries through strict sanitary measures (elimination of stray dogs, destruction of suspected animals, requirements for wearing muzzles on dogs, tracking the movement of rabid dogs and their contacts, restriction of movement and quarantine) (Cliquet et al., 2014; Müller & Freuling, 2020). Therefore, the spread of rabies by reservoir species is a unique problem. Thus, mathematical and simulation models are useful tools to provide an understanding of the most effective rabies control strategies (Elmore et al., 2017; Rattanavipapong et al., 2019).

Analysis of recent researches and publications

Modelling provides an inexpensive way of studying the main parameters of wildlife rabies transmission, rabies management economics, its prevention and control (Sterner & Smith, 2006). Many existing models that study the spread of rabies virus in Europe are associated with the appearance of rabies in one species, namely the red fox (Borchering et al., 2012). However, in Eastern Europe, especially in Ukraine, rabies continues to spread equally through foxes and domestic carnivores (Makovska et al., 2020). As reported by Drozhzhe (2015), over the past decade, our country, together with neighbouring countries such as the Russian Federation, Republic of Belarus, and Romania, has formed the epicentre of rabies, which accounted for up to 90% of all European countries.

In addition, Ukraine is the only country in Europe where rabies is spread even more through domestic carnivores than through foxes (Tkachenko et al., 2014; Yarchuk et al., 2015; Golik et al., 2018). This epizootic situation is provoked on the one hand by a large number of homeless and stray dogs and cats, which move freely in all regions, transporting the causative agent of rabies and exacerbating the epizootic and epidemic situation and causing economic damage (Gulyukin et al., 2016; Kurtyak, 2017). On the other hand, the reason for the high incidence of rabies among domestic carnivores is the low vaccination rate (Achilov & Nedosekov, 2013; Polupan et al., 2019). Because unvaccinated and stray animals are currently potential carriers of the rabies virus, they exacerbate an epizootic situation of an anthropological nature ("urban") (Rebenko et al., 2019).

According to Levkivsky et al. (2016), there is a relationship between the rabies incidence in foxes and stray cats. Because cats often come into contact with foxes during hunting, they become infected and transmit virulent strains of the rabies virus to other domestic carnivores and farm animals. This is facilitated by both the biological characteristics of animals and the intervention of human agricultural activities, which leads to the synantropization of wild animals and the savagery of domestic animals (Perotskaya, 2011). And, if the situation with wild animals is more controlled, then with domestic animals, and even more with stray and homeless animals in cities, the situation is the most unstable, uncontrolled, and threatening, both for humans and animals (Rebenko et al., 2019).

During the geographic information systems analysis for 2018 in all regions of Ukraine, it was found that cases of rabies in domestic carnivores were registered throughout the country with the most concentrated in the east and centre, while for foxes the most affected ar-

eas by rabies were the territories in the west of Ukraine. In general, most cases of rabies were registered in Vinnytsia Oblast. On the one hand, it is the result of a large number of foxes and domestic carnivores, but on the other hand, it is the result of quality monitoring in this area. Also, it was found that during 2018, cases of rabies in foxes registered the most in the first and fourth quarters, and in domestic carnivores mainly in the first quarter (Makovska, 2020).

In most cases, the transmission of the pathogen is mainly a localized process that occurs between animals with the most intense contact, provided that they are in one place. In this case, the movement of animals between settlements contributes to the geographical spread of infectious diseases. Given the limited knowledge about the patterns of animals' movement and their interspecific interaction, it is usually impractical to model each animal within a population.

Therefore, today, the analysis of the spread of rabies in space is possible with the use of appropriate information and analytical technology (Dzyublyk et al., 2017).

Purpose. Thus, the purpose of this study was to model the dynamics of the spread of rabies in areas with the highest concentration of registered rabies cases in the period from 2012 to 2018.

Materials and methods of research

Approximation of percentage values of the dynamics of rabies spread was carried out for the period from 2012 to 2018, taking into account the full set of data collected from all regions of Ukraine. The main parameter of the model – the parameter of rabies transmission, was determined using official epidemiological data from regional laboratories of veterinary medicine and regional departments of the State Service of Ukraine On Food Safety and Consumer Protection.

The development of a qualitative prognostic model for the spread of rabies among domestic and wild animals is impossible today due to the incomplete or contradictory retrospective data on the prevalence of rabies. Accordingly, it is possible to build a concept of information and analytical technology aimed at analysing and summarizing existing data in order to study trends and make appropriate management decisions based on them.

To study and analyse the dynamic spread of rabies in time and space, information and analytical technology was developed, which is based on retrospective data on the recording of rabies cases in certain regions.

Algorithm of work of the developed information-analytical technology:

1. Filter available data according to specified criteria (such as region or species).
2. Clustering of filtered data by time periods in order to obtain data on the area of infection for a certain time period.
3. Sort clusters by time from the first to the last.
4. Determination of the centres of the regions as the average value of the cluster points.
5. Comparison of the obtained clusters with neighbouring ones to obtain data on the distance to which the region of infection has moved. The comparison is made using the geographical coordinates of the points of infection detection.
6. Construction of a discrete distribution of distances for each pair of comparable clusters.

By analysing the obtained discrete distributions of distances over a time pe-

riod, it is possible to determine trends in the distance to which the infection zone is shifted, and based on the analysis of cluster centre displacement, trends in the direction of the infection zone might be determined (Fig. 1).

The set of analysed rabies cases is single-layered, but there are many complex real-world systems that might be represented as several layers of such separate sets. Most complex physical, social, engineering, information, and biological systems include several subsystems and levels of communication, and they are often open, meaningful, oriented, multilevel, multicomponent, rebuilt systems of systems, and located in unstable and changing conditions.

The following indicators: the interval duration of one month and the distance of 20 km, 50 km, and 100 km were used for the analysis. Data were entered separately for each rabies case in the format of the region, district, city or town, animal species, date of diagnosis confirmation, and geographical coordinates of latitude and longitude.

The diagram was built in draw.io (https://app.diagrams.net/).

The limitation of the research was the limited method for modelling in areas with a small number of cases, as a result of

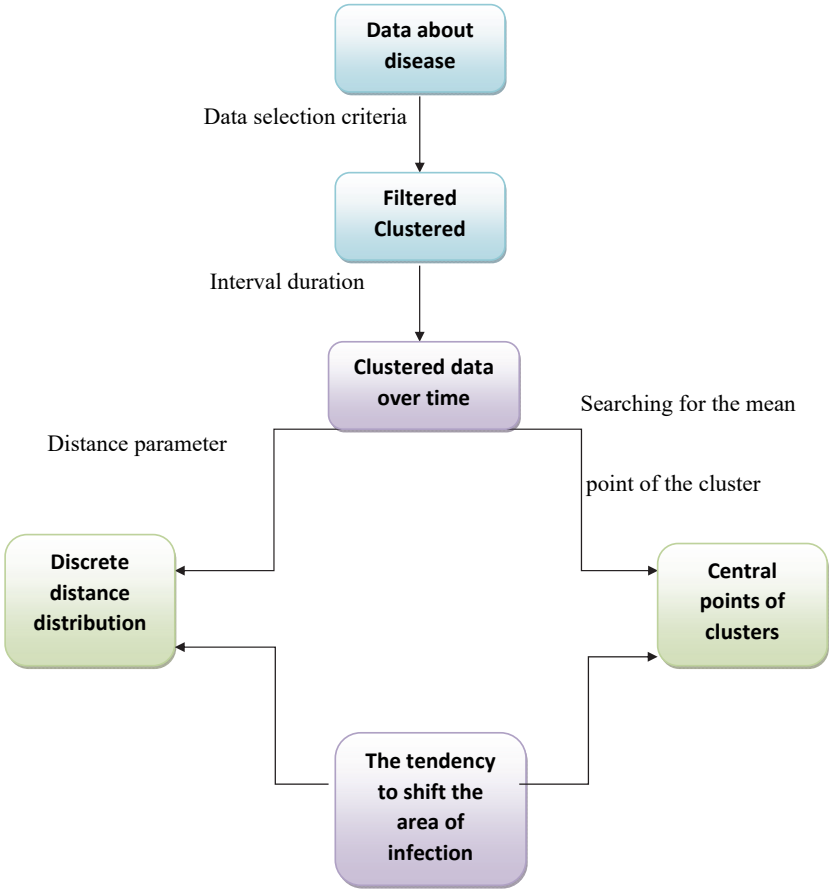
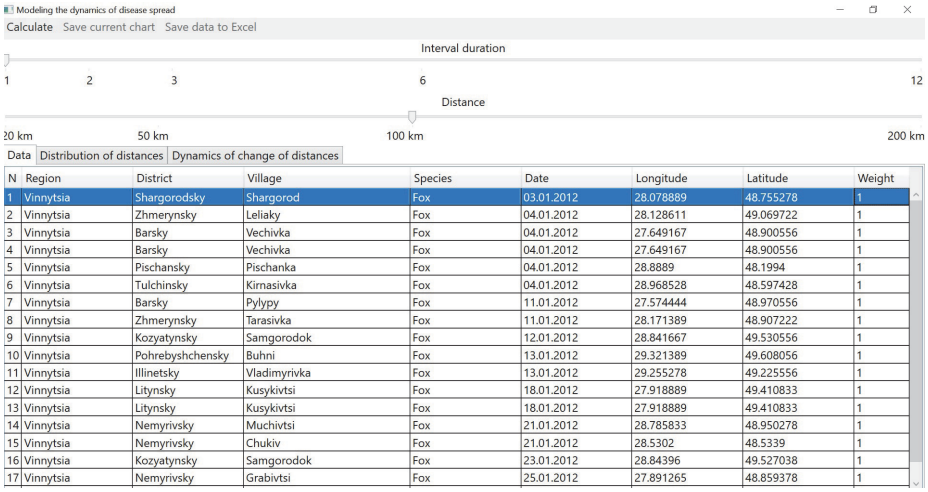


Fig. 1. Algorithm of actions of the developed information-analytical technology



N	Region	District	Village	Species	Date	Longitude	Latitude	Weight
1	Vinnytsia	Shargorodsky	Shargorod	Fox	03.01.2012	28.078889	48.755278	1
2	Vinnytsia	Zhmerynsky	Leliaky	Fox	04.01.2012	28.128611	49.069722	1
3	Vinnytsia	Barsky	Vechivka	Fox	04.01.2012	27.649167	48.900556	1
4	Vinnytsia	Barsky	Vechivka	Fox	04.01.2012	27.649167	48.900556	1
5	Vinnytsia	Pischansky	Pischanka	Fox	04.01.2012	28.8889	48.1994	1
6	Vinnytsia	Tulchinsky	Kirnasivka	Fox	04.01.2012	28.968528	48.597428	1
7	Vinnytsia	Barsky	Pylypy	Fox	11.01.2012	27.574444	48.970556	1
8	Vinnytsia	Zhmerynsky	Tarasivka	Fox	11.01.2012	28.171389	48.907222	1
9	Vinnytsia	Kozyatynsky	Samgorodok	Fox	12.01.2012	28.841667	49.530556	1
10	Vinnytsia	Pohrebyshchensky	Buhni	Fox	13.01.2012	29.321389	49.608056	1
11	Vinnytsia	Illinetsky	Vladimyrivka	Fox	13.01.2012	29.255278	49.225556	1
12	Vinnytsia	Litynsky	Kusykivtsi	Fox	18.01.2012	27.918889	49.410833	1
13	Vinnytsia	Litynsky	Kusykivtsi	Fox	18.01.2012	27.918889	49.410833	1
14	Vinnytsia	Nemyrivsky	Muchivtsi	Fox	21.01.2012	28.785833	48.950278	1
15	Vinnytsia	Nemyrivsky	Chukiv	Fox	21.01.2012	28.5302	48.5339	1
16	Vinnytsia	Kozyatynsky	Samgorodok	Fox	23.01.2012	28.84396	49.527038	1
17	Vinnytsia	Nemyrivsky	Grabivtsi	Fox	25.01.2012	27.891265	48.859378	1

Fig. 2. Sample data on rabies cases among foxes for the 7-year period (2012–2018) in Vinnytsia Oblast

which the results of modelling in areas with the highest concentration of rabies cases were taken into account, the epicentre of which in recent years is Vinnytsia Oblast. Thus, we conducted modelling with data from all regions of Ukraine, but this paper presents the results on the example of Vinnytsia Oblast. For analysis, the developed system included information from the national database created by us, which was adapted for the modelling (Fig. 2).

Results of the research and their discussion

These results carry the greatest prognostic information about the peculiarities of the epizootic process of rabies. Modelling the dynamics of rabies in Ukraine differs as in territorial features as in species and season. Thus, we found that the disease spread up to 250 km per month within the region.

In general, the distance of distribution of rabies depended on season and species of animals. Firstly, we conducted modelling for 7 years. The results of modelling the spread of rabies with fixed intervals of up to 20 km and the interval duration of one month on the example of Vinnytsia Oblast are presented in Figure 3.

As we can see from this graph, the movement of rabies in foxes, dogs and cats has a wavy dynamics and is repeated every year with varying intensity. In summary, animals transmitted rabies over short distances in 10–40% of cases. Distribution of rabies among species has shown that wild animals transmit the disease mainly over longer distances, which agrees with the available literature data.

Besides this, we conducted modelling of the dynamics of distance change at a fixed distance of up to 100 km and the interval duration of one month, which is presented in Figure 4.

These curves show the peaks of spread over long and short distances within each month. Among foxes the reduction of all peaks for the period from August 2015 to August 2016 is significant. This can be explained by changes in the number of foxes and the presence of a forage bases in this period.

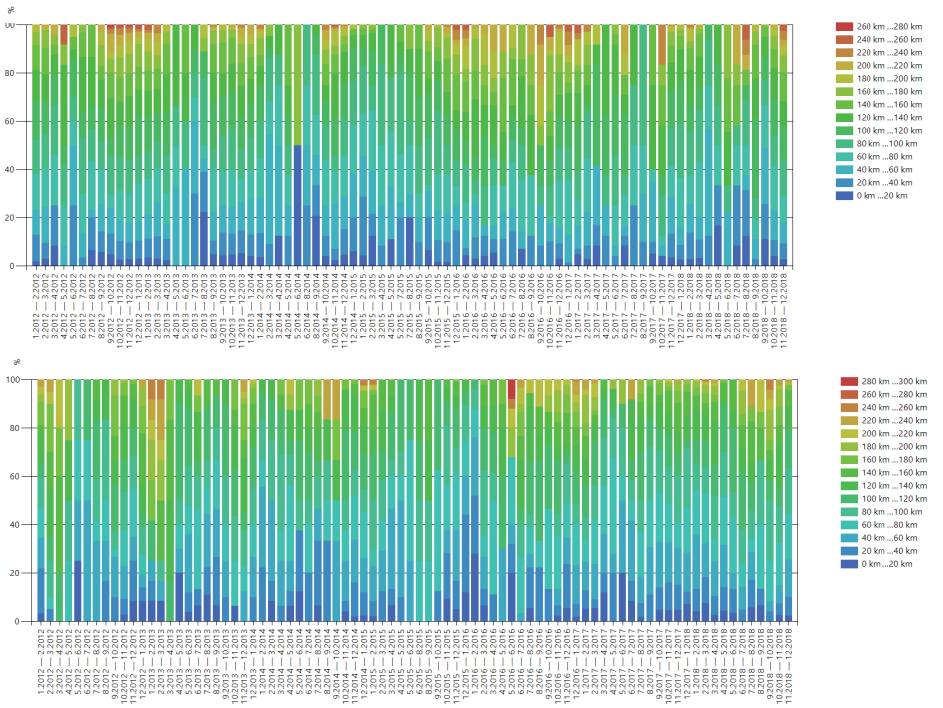


Fig. 3. Dynamics of distribution of rabies for the period from 2012 to 2018 on the territory of Vinnytsia Oblast (A – among foxes, B – among domestic carnivores)

For a more detailed study, we set aside one year and determined the distribution of rabies separately for foxes and domestic carnivores (Fig. 5).

These curves show sharp changes in the dynamics of rabies spread among foxes, and smoother for cats and dogs. This is due to the possibility of domestic carnivores being more attached to one habitat (Rebenko et al., 2019).

In addition, the longest periods of movement of rabid foxes were registered from July to August and from November to December in 2018. And among domestic carnivores, there is a clear visual rise of long-distance movements in the summer-autumn period and a decline in the winter-spring period.

Based on the results presented in the graphs, we determined the percentage of the dispersion of rabies, which is presented in Table 1.

To build this model, 95 confirmed cases of rabies among dogs and cats (domestic and stray) were studied. From the results presented in the graph and in Table 1, we can see that the dynamics of the distribution of cases differed significantly depending on seasonality. Thus, we found that rabies spread the most at a distance of up to 50 km in the winter (26.45%) compared to other months, at a distance from 50 to 100 km – in the autumn (40.33%), and at a distance of more than 100 km – in the summer (49.84%).

In general, domestic carnivores were sources of rabies within a radius of 50 km

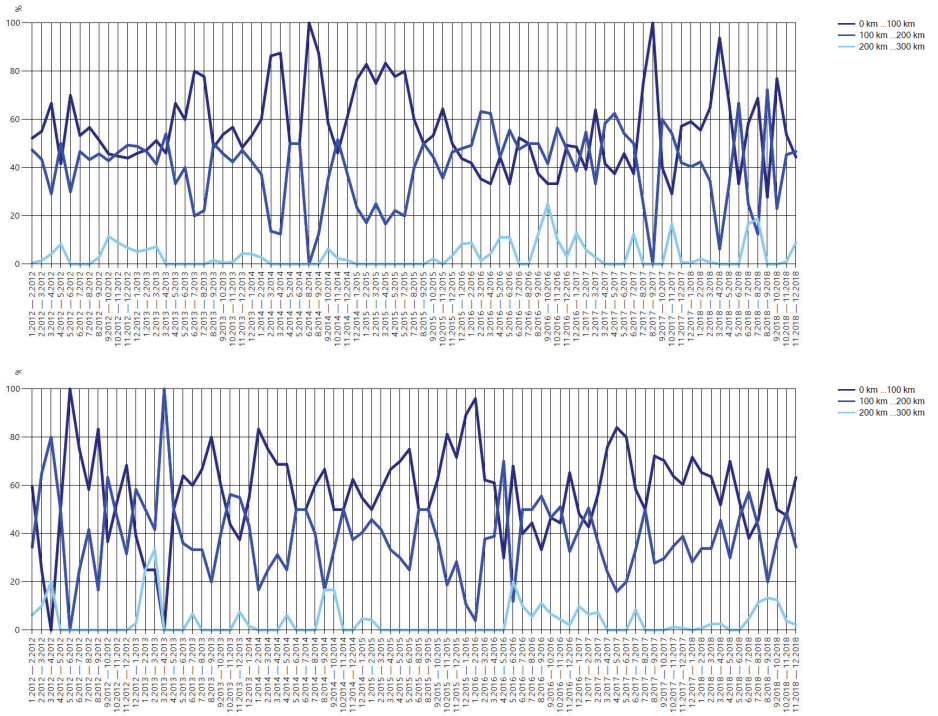


Fig. 4. Dynamics of distance change of rabies cases for the period from 2012 to 2018 on the territory of Vinnytsia Oblast with the fixed distance of up to 100 km (A – among foxes, B – among domestic carnivores)

in only 16.98 % of cases, and within a radius of 100 km – in 55.95% of cases.

Although this issue has not been studied in Ukraine today, there is an opinion in rabiology that a certain species of animals can carry the disease to a specific distance. As reported by Shcherbak (1982), the concept of ecological and anthropological limited borders of the spread of the disease means the migratory resources of animals, namely: wolf – up to 150 km, fox – 10–15 km, dog – 5 km, marten – 3 km. That is the distance within which one infected animal can transmit the virus to another animal or human.

In our study, we found that domestic carnivores can carry the rabies virus over long distances (up to 100 km) during the month.

This could be explained by the inclusion into the epizootic process not only of domestic dogs and cats, which have owners and should be vaccinated, but also homeless and stray dogs and cats that usually are not vaccinated and can move freely over long distances, and in an aggressive form of the disease to attack oncoming animals and people on their way.

This statistic is confirmed by Kornienko et al. (2019), where on the example of the analysis of national data for 2018, it was found that 253 people were bitten by vaccinated stray dogs, and 8 788 people (97.20%) were affected by unvaccinated stray dogs. If we use the data only for the Vinnytsia Oblast, 98 bites were inflicted by vaccinated stray

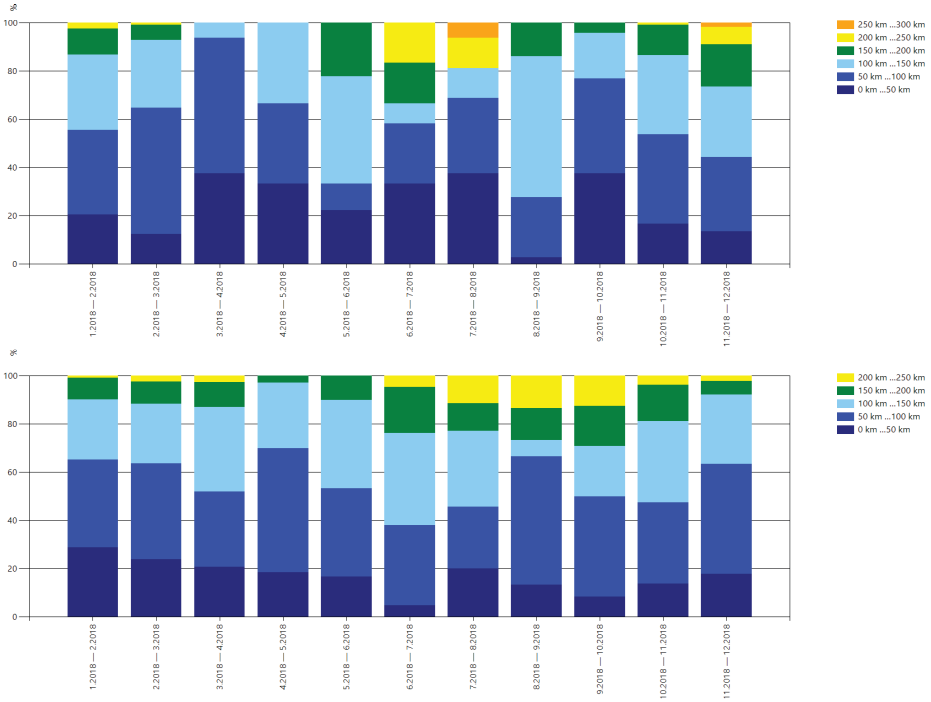


Fig. 5. Dynamics of the distribution of rabies for 2018 on the territory of Vinnytsia Oblast (A – among foxes, B – among domestic carnivores)

dogs and 199 on unvaccinated stray dogs, i.e., twice as many.

It should be noted that even domestic carnivores, which had a host in 80%, were not vaccinated against rabies (Mazur et al., 2017). Also, the high incidence among domestic carnivores, especially cats, indicates a lack of regulation of their number in cities and villages, as well as the lack of a proper number of routine vaccinations among animals of these species (Titarenko, 2019).

When studying the seasonal dynamics, it can be assumed that in winter domestic carnivores transmitted the rabies virus mainly over short distances due to adverse weather conditions that limited movement in general, and in summer this trend reversed and the disease spread to the longer distances due to favourable weather conditions, biological characteristics of cats that are prone to straying (Makovska et al., 2018), and also due to stray animals (Levkivsky et al., 2016).

In order to build the model for modelling dynamics of rabies spread in foxes, 145 confirmed cases of rabies among foxes were studied. The level of dispersion of disease is presented in Table 2.

Thus, we found that rabies spread at a distance of up to 50 km the most in the spring (31.01%) compared to other months, at a distance from 50 to 100 km – in the winter (43.67%), and at a distance of more than 100 km – in the summer (48.38 %), similar to the indicators of domestic carnivores. In general, foxes were sources of rabies within a radius of 50 km in only 24.33% of cases and within a radius of 100 km – in 58.54% of cases.

1. Indicators on the dynamics of the distribution of rabies by domestic carnivores for 2018 in Vinnytsia Oblast

Month	Distance, km							
	<50	Mean value	50–100	Mean value	<100	Mean value	>100	Mean value
	The level of the dispersion of disease,%							
January	28.93	26.45	36.36	38.01	65.29	64.46	34.71	34.53
February	23.97		39.67		63.64		34.36	
March	20.78	18.67	31.17	39.75	51.95	58.43	48.05	41.57
April	18.57		51.43		70.00		30.00	
May	16.67		36.67		53.34		46.66	
June	4.76	12.60	33.33	37.45	38.09	50.15	61.91	49.84
July	20.00		25.71		45.71		54.29	
August	13.33		53.33		66.66		33.34	
September	8.33	13.28	41.67	40.33	50.00	53.61	50.00	46.38
October	13.75		33.75		47.50		52.50	
November	17.78		45.56		63.34		36.66	
Mean value	16.98		38.96		55.95		43.86	

2. Indicators on the dynamics of the distribution of rabies by foxes for 2018 in Vinnytsia Oblast

Month	Distance, km							
	>50	Mean value	50–100	Mean value	>100	Mean value	<100	Mean value
	The level of dispersion of disease,%							
January	20.62	16.56	35.00	43.67	55.62	60.23	44.38	39.77
February	12.50		52.34		64.84		35.16	
March	37.50	31.01	56.25	33.56	93.75	64.58	6.25	35.42
April	33.33		33.33		66.66		33.34	
May	22.22		11.11		33.33		66.67	
June	33.33	24.53	25.00	27.08	58.33	51.62	41.67	48.38
July	37.50		31.25		68.75		31.25	
August	2.78		25.00		27.78		72.22	
September	37.61	22.62	39.32	35.68	76.93	58.31	23.07	41.68
October	16.64		37.05		53.69		46.31	
November	13.63		30.69		44.32		55.68	
Mean value	24.33		34.21		58.54		41.46	

As mentioned above, according to Shcherbak (1982), the fox's migration resources were 10–15 km. Based on this (taking into account the following criteria), a spatial criteria for foci of fox rabies was established, which included the place of detection of a sick or died from rabies animal, including an area within a radius of 10–15 km. However, our results show significant differences. This can be explained by the ecological characteristics of foxes and the high number of fox populations in today's conditions, which increases the prevalence of rabies in wild animals. It is known that the population is affected by the presence or absence of fodder base, which for foxes consists mainly of rodents, and as more land is ploughed for agriculture, the fodder base increases every year, so the prevalence of this species also increases (Brusentsova, 2019). Accordingly, the larger the animal population, the greater the risk of rabies and the greater the area affected.

If we consider the seasonal aspect, then in our results, the vast majority of foxes carried the pathogen for shorter distances in spring, medium in winter, and the longest in summer. The spread of rabies in the winter at distances from 50 to 100 km might be explained by the need for wild animals to cover long distances in search of prey. Spring and summer trends deserve special consideration, because according to scientists (Golik, 2016; Polupan, 2017) the spring period in foxes is associated with the mating season and they can cover long distances, biting and carrying the causative agent of rabies, and in summer foxes are engaged growing fox cubs and only in the fall disperse throughout the territory. Therefore, this issue requires additional research by both biologists and veterinarians.

Conclusions and future perspectives of the study

In conclusion, modelling the dynamics of rabies in an area with a high density of rabies cases (Vinnytsia Oblast) for 2018 allowed to obtain new data on the spread of rabies through domestic carnivores. It was determined that at a distance of up to 50 km rabies is spread mainly in winter, up to 100 km and more – in summer. In general, domestic carnivores were sources of rabies within a radius of 50 km in only 16.98% of cases, and within a radius of 100 km – in 55.95% of cases. For foxes, the simulation found that up to 50 km foxes transmitted rabies the most in the spring months (31.01%), up to 100 km – in the winter months (43.67%), over 100 km – also in the summer months (in 48.38%).

These results should be taken into account when planning and conducting preventive vaccinations among domestic and wild animals in order to better prevent the spread of rabies, preserve the livestock and humans and eradicate the disease. Prospects for further research will be the forecasting of rabies epizootics in Ukraine.

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Анотація. З*авдяки турботі про охорону здоров'я тварин та людей постійно зростають зусилля щодо розроблення математичних та обчислювальних моделей для прогнозування просторової та часової динаміки інфекційних хвороб, у тому числі і сказу. Тому, нашою метою було провести моделювання динаміки поширення сказу лисицями, собаками та котами на прикладі Вінницької області в період із 2012 до 2018 років. Для дослідження та аналізу динамічного поширення сказу в часі та просторі було розроблено інформаційно-аналітичну технологію,*

яка ґрунтується на основі ретроспективних даних зареєстрованих випадків сказу. Параметр передачі сказу був визначений із використанням офіційних епізоотологічних даних з обласної лабораторії ветеринарної медицини та обласного відділення Держпродспоживслужби України. Виявлено, що сказ серед котів та собак поширювався на відстань до 50 км найбільше (26,45%) у зимовий період, від 50 до 100 км – в осінній період (40,33%), понад 100 км – у літній період (49,84 %). Загалом, домашні м'ясоїдні були джерелами сказу в радіусі 50 км лише в 16,98% випадків, а в радіусі 100 км – уже в 55,95% випадків. Лисиці були джерелами сказу в радіусі 50 км лише у 24,33% випадків, а в радіусі 100 км – уже в 58,54 % випадків. Водночас, найбільше поширення сказу серед лисиць на відстань до 50 км спостерігалось у весняний період (31,01%), від 50 до 100 км – у зимовий період (43,67%), понад 100 км – у літній період (48,38%). Отримані нові дані будуть корисними під час планування та проведення профілактичних антирабічних заходів. У подальшому планується провести моделювання в розрізі всіх областей та створення прогнозів поширення сказу територією України.

Ключові слова: *сказ тварин, моделювання поширення сказу, лисиці, домашні м'ясоїдні, інформаційно-аналітичні технології*

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MICROSCOPIC CHANGES IN LAYING HENS KIDNEYS AT THE EGG DROP SYNDROME

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Abstract. *The article presents the results of studying the microscopic changes in laying hens' kidneys under egg drop syndrome (EDS-76). Clinical signs of EDS-76 in laying hens included decreasing of egg production, suppression, changes in egg pigmentation, laying of small-sized eggs with a soft shell, or without a shell at all. During the dissection enlarged, pale kidneys with clearly dilated blood vessels were registered. The lungs in most cases had an uneven red-pink color. In many laying hens, the spleen had an uneven color – it showed areas of different sizes and shapes of grayish, bluish, and red. In some cases, the liver was unevenly colored (with areas of yellowish and clay color), and in other cases, point and spotted hemorrhages were found under its capsule. However, the most pronounced and permanent gross changes registered in reproductive organs. During the histological examinations of the kidneys of laying hens infected with EDS-76 virus, we found that in this organ, there are specific for this disease and nonspecific microscopic changes, which are registered under the kidney damage of various etiologies. Specific for EDS-76 changes include the presence of basophilic and eosinophilic inclusion bodies in the nuclei of many epithelial cells in convoluted tubules, part of epithelial cells of straight tubules, and part of endothelial cells, podocytes, and mesangiocytes of the many glomeruli. Some of nuclei completely acquired distinctly oxyphilic properties. Nonspecific microscopic changes included diverse changes in nuclei and cytoplasm in epithelial cells of convoluted and straight tubules, violations of intercellular junctions, damages of renal corpuscles.*

Keywords: *laying hens, egg drop syndrome, clinical signs, gross changes, kidneys, microscopic changes.*

Introduction

Broiler meat and chicken eggs are cheap foods. Feed costs for protein and energy production in poultry are the lowest compared to other livestock industries. The production of 1 g of pure protein in eggs and meat or poultry feed units is 8 times less than in beef, 3 times less than in pork. It takes 12 times less time to produce 1 ton of meat than in cattle breeding, and 8 times less than in pig breeding (Vermienko, 2008; Nazarenko, 2009). One egg satisfies a human daily need for vitamin B2 by 10–12%, vitamin D – by 10–40%, vitamin A – by 15–16% (Oshchipok, 2007).

Various infectious and non-infectious diseases cause great problems for poultry (Giasuddin et al., 2002). Infectious diseases mainly cause decreased egg production in hens. These diseases include infectious bronchitis, Newcastle disease, infectious encephalomyelitis, avian pox, 4th serotype of chicken adenovirus group I and the egg drop syndrome (Badar et al., 2006; Geetha et al., 2008). The egg drop syndrome (EDS-76) is primarily a significant economic problem due to product shortages and additional costs for diagnostic and prevention (Begum et al., 2013).

EDS-76 is an infectious viral disease in laying hens, characterized by a sharp decrease in egg production, damage of the reproductive organs, changes in pigmentation and egg shape, thinning and deformation of the shell, reduced protein value, reduced percentage of hatching chicks from hatching eggs and reduced viability of chicks (Anufrieva & Olshevskaya, 1992; McNulty & Smyth, 2002).

In addition to chickens, EDS-76 registered in quail, pheasants, and guinea fowl. Turkeys can be infected experimentally, but they do not show clinical signs (Parsons et al., 1980). Antibodies to EDS-76 virus were found in a large number of bird species: domestic and wild ducks and geese, herons, seagulls, owls, storks and swans and pigeons (Schloer, 1980; Watanabe & Ohmi, 1983; Das & Pradhan, 1992; Bidin et al., 2007; Ezema et al., 2010).

Until 2001, it was thought that EDS-76 virus was not virulent to ducks and geese. However, in 2001, this virus was isolated at the outbreak of the respiratory disease in young goslings, and the disease was reproduced experimentally (Ivanics et al., 2001).

In many countries, EDS-76 virus infects up to 50 % of chicken flocks (Badar et al., 2006). Chickens of any age and all breeds and crosses are susceptible to this infection (Zsak & Bartha, 1979; Al-Hilly et al., 1982; Curtis & Boachie, 1982; Howell, 1982; Ibrahimov & Osidze, 1988; McFerran & Smyth, 2000; Elayo et al., 2010).

Infection can occur at any age, but the clinical signs of the disease are limited to the period of egg production, which is probably due to the reactivation of the latent virus (McFerran et al., 1978; Kaleta et al., 2003; Suresh et al., 2013).

Pathomorphological changes under EDS-76 are only superficially described in only a small number of original researches (Bakulin et al., 1988; Van Eck et al., 1976; Taniguchi et al., 1981; Brugh et al., 1984; Chetty et al., 1987; Ivanics et al., 2001).

According to McFerran and Smyth (2000), pathognomonic changes in laying hens are short-lived and are present only during the period of falling egg production.

According to many authors, pathomorphological changes are generally minimal and limited by the reproductive tract of laying hens. Inactive ovaries,

oviduct atrophy, edema, and white exudate in the uterus may be registered. In the uterus – dystrophic changes and desquamation of epithelial cells, glandular atrophy, infiltration by heterophiles, lymphocytes, and plasma cells. In the epithelial cells of the uterus, cervix, and vaginal area – intranuclear inclusion bodies (Taniguchi et al., 1981; Brugh et al., 1984; Chetty et al., 1987).

Bakulin et al. (1988) in addition to changes in the reproductive tract of laying hens infected by the EDS-76 virus, found enlarged kidneys, numerous hemorrhages under the liver capsule, atrophy of the cloacal bursa and spleen, and the accumulation of exudate in the pericardium and intramuscular connective tissue. EDS-76 virus is also minimally replicate in the gastrointestinal tract (McFerran, 2000; Smyth, 2000).

Materials and methods of research

The research was performed at the poultry farm "Khorostpodillya" of the Khmelnitskyi region on laying hens of "Hisex White" and "Hisex Brown" crosses. The diagnosis on EDS-76 was established by epidemiological data, clinical signs, and results of the dissection and laboratory researches. Laboratory researches (ELISA-test) was performed in the Center for Veterinary Diagnostics (Kyiv).

Dissection of 28 laying hens (11 laying hens of the cross "Hisex White" and 17 laying hens of the cross "Hisex Brown") was performed by the method of partial evisceration in the conventional sequence (Zone et al., 2009). During the dissection, the pieces from different parts of the kidneys (at least 3 pieces from each kidney) were selected for histological examination

Pieces of the kidney were fixed in 10% aqueous formalin solution with a pH 7.2–7.4, dehydrated in ethanol of increasing concentration, and through chloroform embedded into paraffin. Slides with a thickness of 8 ± 1 μm were stained with hematoxylin and eosin (Goralsky et al., 2005). All steps were carried out at room temperature. The slides were studied under a light microscope Micros 100LED (Austria) at magnifications of x 50–1500. Photographing of slides was performed through a photographic nozzle NDPL-2(2X) using a camera Canon EOS 550D.

Results of the research and their discussion

Clinical signs of EDS-76 in laying hens included decreasing of egg production, suppression, changes in egg pigmentation, laying of small-sized eggs with a soft shell, or without a shell at all.

During the dissection of laying hens with EDS-76 registered enlarged, pale kidneys with clearly dilated blood vessels. The lungs in most cases had an uneven red-pink color. In many laying hens, the spleen had an uneven color – it showed areas of different sizes and shapes of grayish, bluish, and red. In some cases, the liver was unevenly colored (with areas of yellowish and clay color), and in other cases, point and spotted hemorrhages were found under its capsule.

However, the most pronounced and permanent changes registered in reproductive organs. In some laying hens, gross changes in the ovaries and oviduct were not detected, except for hyperemia in individual oocytes at the early stages of development.

In most laying hens, the redness of the vast majority, or all oocytes, and inflammation of the oviduct was marked. In the

lumen of the caudal part of the oviduct were often formed eggs with a soft shell, which easily burst at low pressure on it. The protein of such eggs was turbid.

In some laying hens, egg formation completely stopped in the early stages. This was evidenced by the complete absence of oocytes in the late stages of formation and the absence of formed eggs in the caudal part of the oviduct. In some laying hens, signs of egg maturation and egg formation were not detected at all. The tendency to reduce the weight of the whole oviduct or its individual parts was detected in laying hens with EDS-76.

In addition to these signs, in some laying hens was the serous catarrh of the small intestine, as well as atrophy of the cloacal bursa and spleen.

During the histological examinations of the kidneys of laying hens infected with EDS-76 virus, we found that in this organ, there are specific for this disease and nonspecific microscopic changes, which are registered under the kidney damage of various etiologies.

Specific for EDS-76 changes include the presence of basophilic and eosinophilic inclusion bodies in the nuclei of many epithelial cells in convoluted tubules, part of epithelial cells of straight tubules, and part of endothelial cells, podocytes, and mesangiocytes of many glomeruli (Fig. 1–3). Some of the nuclei completely acquired distinctly oxyphilic properties (Fig. 1).

Previously, in a cell culture model found that virus particles and four types of virus-induced inclusions appeared in the nucleus in 24 hours after infection (Adair et al., 1979). The dynamics of their development is similar to that of avian adenoviruses of subgroup I (Mar-

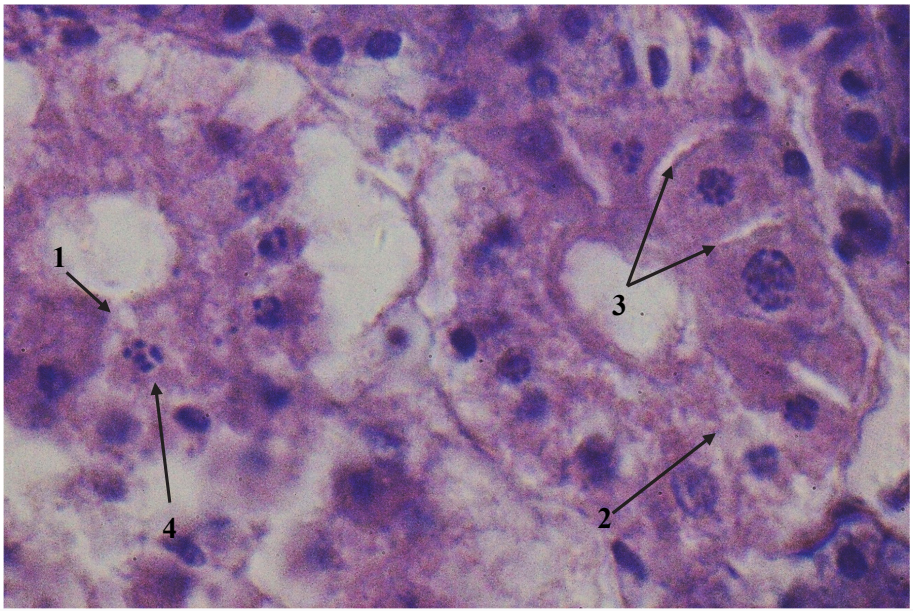

Fig. 1. Convoluted tubules in the kidney of a laying hen under EDS-76:
1 – lysis of the apical cytoplasm of the epithelial cell; 2 – lysis of all parts in the cytoplasm of the epithelial cell; 3 – discomplexation of neighboring epithelial cells; 4 – basophilic inclusion bodies in the eosinophilic nucleus of the epithelial cell. Hematoxylin and eosin, x 1000

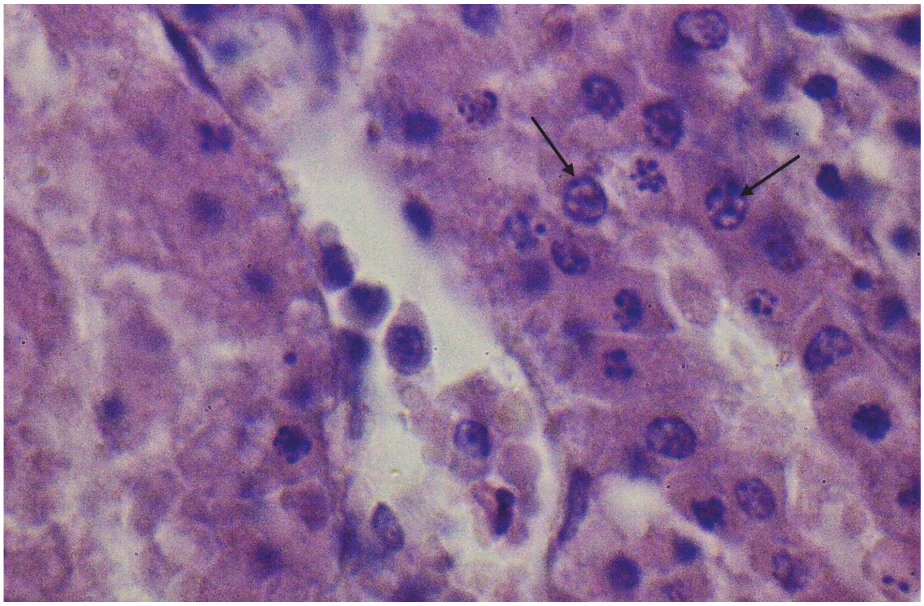

Fig. 2. Convoluted tubules in the kidney of a laying hen under EDS-76: eosinophilic inclusion bodies in the nuclei of epithelial cells (arrows). Hematoxylin and eosin, x 1000

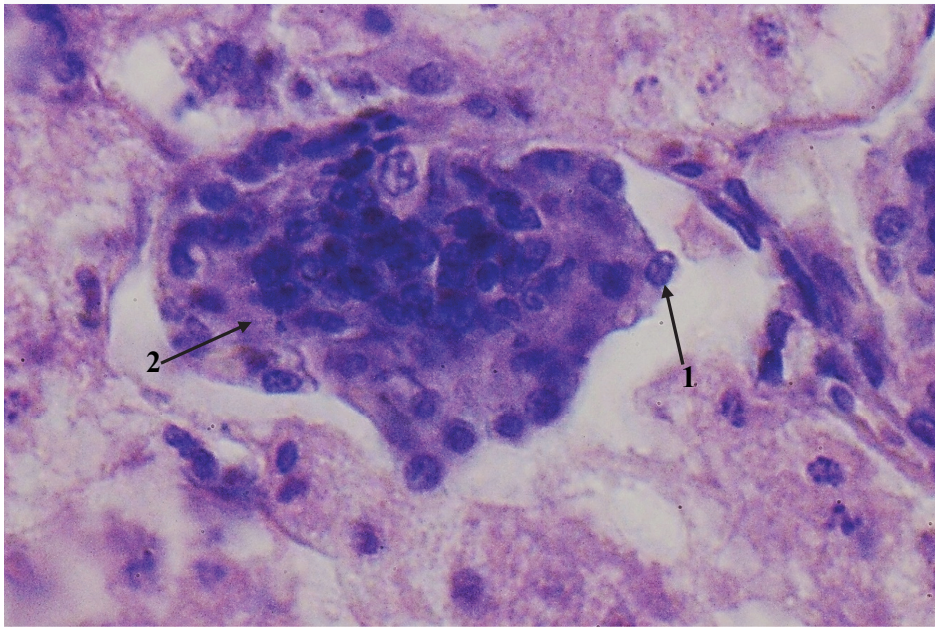

Fig. 3. Renal body of a laying hen under EDS-76: 1 – eosinophilic inclusion bodies in a nucleus of a mesangiocyte; 2 – eosinophilic substance. Hematoxylin and eosin, x 1000

tinez-Palomo et al., 1967; Adair et al., 1978; Adair et al., 1979).

Under EDS-76 the intranuclear inclusion bodies are found in epithelial cells of the uterus, a cervix uterus, and a vaginal site (Taniguchi et al., 1981; Brugh et al., 1984; Chetty et al., 1987).

Staining of monolayer cells with hematoxylin and eosin can be used to control EDS-76 virus replication in cell culture. The first changes are detected after 24 hours. They manifest as several eosinophilic inclusion bodies in the nuclei of infected cells. Later, one or two large inclusion bodies were found in the nucleus, which consists of separate clusters. The nucleoplasm around the inclusion bodies acquires a granular structure, separates from the nuclear envelope and nucleolus, and forms one or more basophilic pockets, separated by unpainted areas (Adair et al., 1979).

It was also found, that kidney and liver trypsinized cell cultures can be used to isolate EDS-76 virus. The virus reproduces the best in duck kidney cell culture, worse – in duck and chicken liver cell cultures, and very poorly – in turkey kidney and liver cell cultures (Adair et al., 1979).

Considering the literature, we believe that we found that intranuclear inclusion bodies in various kidney cells reflect replication of EDS-76 virus in them. In the convoluted tubules, basophilic inclusion bodies were found in nuclei of many epithelial cells. The number of such inclusion bodies in nuclei of epithelial cells was different – from 4 to 14.

The size of inclusion bodies was small – from 0.5 to 2.7 μm. The shape of intranuclear basophilic inclusion bodies was also different. Most of them

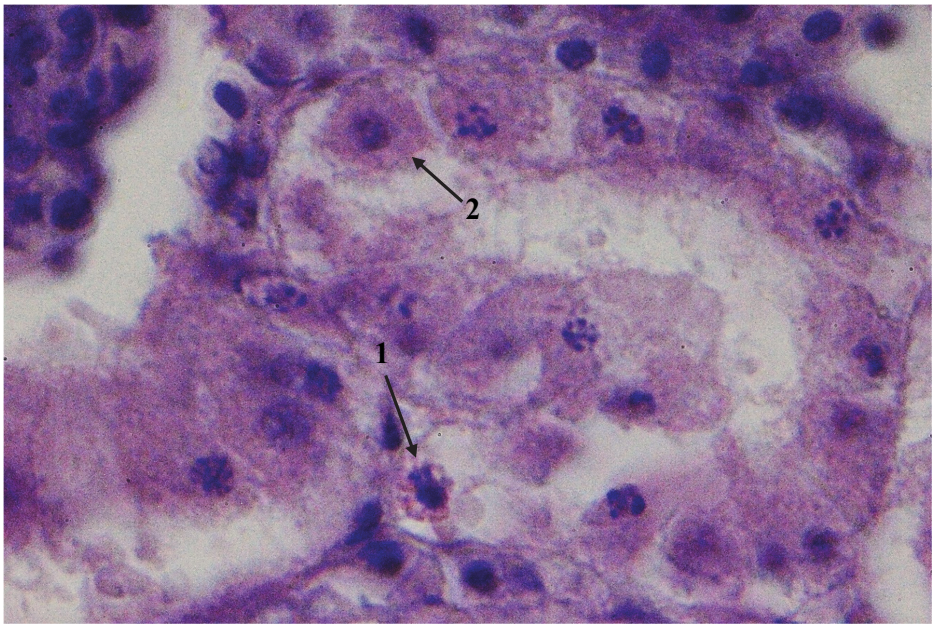

Fig. 4. Convoluted tubules in the kidney of a laying hen under EDS-76:
1 – basophilic inclusion bodies in the cytoplasm of the epithelial cell; 2 – round-shaped epithelial cell. Hematoxylin and eosin, x 1000

were round or oval in shape, but in some nuclei, there were inclusion bodies of elongated and irregular shapes.

In some epithelial cells, basophilic inclusion bodies were found in the cytoplasm (Fig. 4). With the destruction of epithelial cells, basophilic inclusion bodies remained among the cell detritus, and with complete lysis of the cells, they lay freely in the lumen of the tubule.Basophilic inclusion bodies from the chromatin of the nucleus were well-differentiated based on the following features. First, the inclusion bodies were much more intensely stained in blue color with hematoxylin compared to chromatin. Second, inclusion bodies remained basophilic even when all other parts of the nucleus acquired oxyphilic properties. Third, basophilic inclusion bodies remained intact even after the complete lysis of epithelial cells.

In convoluted tubules were also found non-specific for EDS-76 changes. Epithelial cells of all convoluted tubules were in a state of cell swelling. As a result, their size significantly increased. In the part of the convoluted tubules, the increase in the volume of epithelial cells was so significant that the lumen of the tubule completely or almost completely disappeared (Fig. 5).

In some epithelial cells, the cytoplasm lysis was observed. Cytoplasmic lysis is registered in different areas of epithelial cells: basal, apical, and perinuclear (Fig. 1, 5). However, most of the cytoplasm in many cases was lysed in the basal part of the cells.

Complete or partial discomplexation of adjacent altered epithelial cells of convoluted tubules was detected in some places (Fig. 1). At the same time, part of the discomplexed epitheliocytes

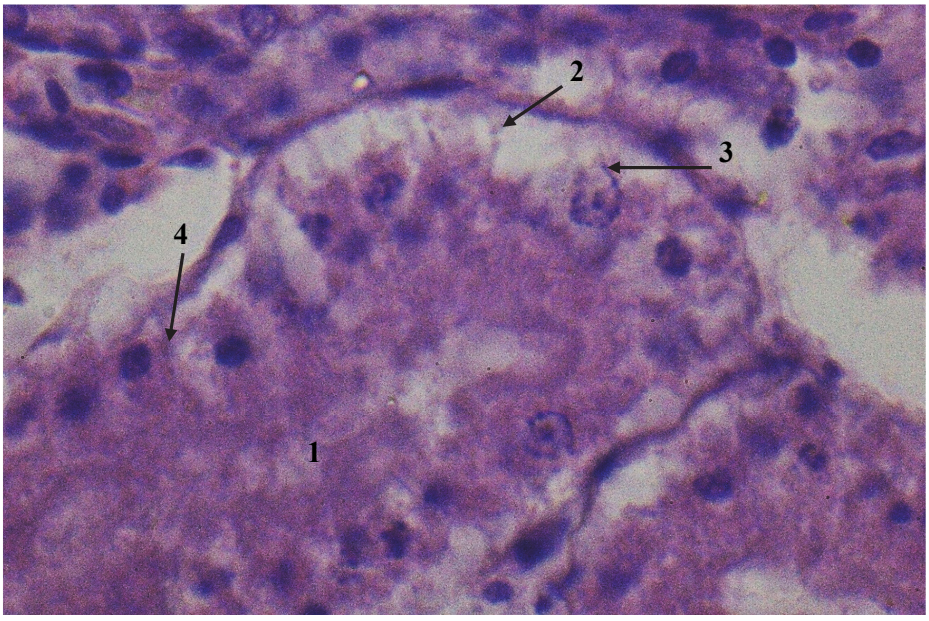

Fig. 5. Convoluted tubules in the kidney of a laying hen under EDS-76:
1 – lack of tubular lumen; 2 – lysis of the basal part of cytoplasm in the epithelial cell; 3 – lysis of the nuclear envelope in the epithelial cell; 4 – cell swelling. Hematoxylin and eosin, x 1000

acquired a rounded shape (Fig. 4). Part of the epithelial cells was separated from the basal membrane into the lumen of the tubule.

In many tubules, there is pronounced subepithelial edema, as a result of which all epithelial cells were separated by a continuous layer from the basal membrane (Fig. 6).

In a part of the epithelial cells of the convoluted tubules, changes in shape, increase in the size of the nucleus, and the presence of protrusions of the nuclear envelope were registered. Partial lysis of the nuclear envelope and nucleus of epithelial cells was also observed (Fig. 5).

In the straight tubules in the kidneys of laying hens with EDS-76, the microscopic changes were slightly different from similar changes in the convoluted tubules. Signs of cell swelling and partial lysis of the cytoplasm were registered in their epithelial cells. In many areas, partial or complete discomplexation of neighboring epithelial cells and their complete or partial separation from the basal membrane were detected.

Hyperchromatosis of the nuclear envelope was registered in the nuclei of some cells. Part of the epithelial cells of the straight tubules were destroyed. In some cells, karyorrhexis was detected in some cases. Intranuclear inclusion bodies in straight tubules were found only in individual epitheliocytes.

Many renal corpuscles had irregular shapes. In glomeruli registered proliferation of mesangiocytes. Sometimes registered a significant expansion of the exit of the proximal convoluted tubule from the renal corpuscle (Fig. 6).

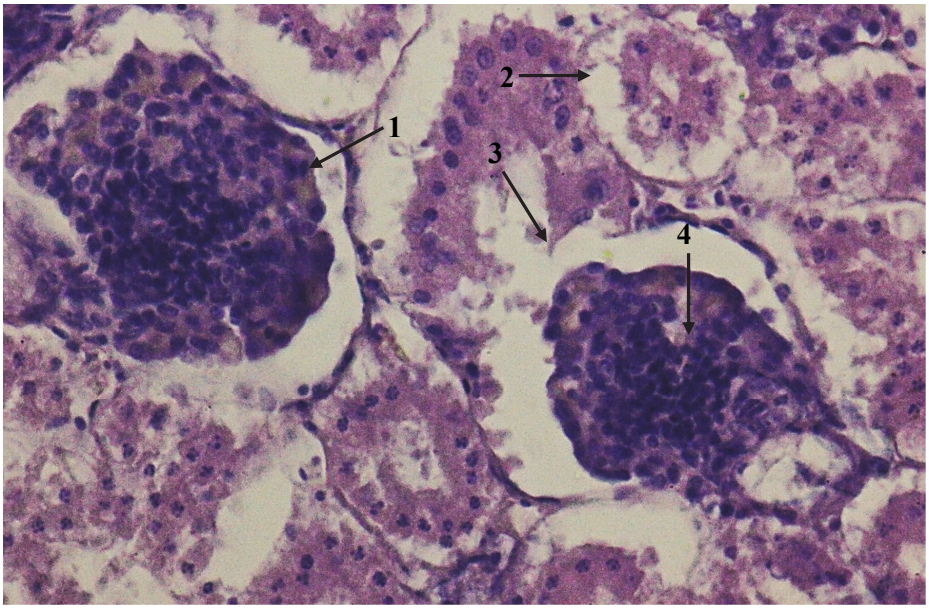

Fig. 6. The kidney of a laying hen under EDS-76: 1 – renal corpuscle of irregular shape; 2 – subepithelial edema; 3 – significant expansion of the exit of the proximal convoluted tubule from the renal corpuscle; 4 – proliferation of mesangiocytes. Hematoxylin and eosin, x 200

Conclusion

In the kidneys of laying hens under EDS-76 were registered specific and nonspecific changes. Specific changes should be considered in the pathomorphological diagnosis of EDS-76.

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Анотація. *У статті представлені результати дослідження мікроскопічних змін нирок курей-несучок за синдрому зниження несучості (СЗН). Клінічні ознаки СЗН у курей-несучок включали зниження несучості, придушення, зміни пігментації яєць, знесення яєць невеликих розмірів з м'якою оболонкою або взагалі без шкаралупи. Під час розтину реєструються збільшені бліді нирки з чітко розширеними кровоносними судинами. Легені здебільшого мали нерівномірний червоно-рожевий колір. У багатьох курей-несучок селезінка мала нерівномірний колір – у ній виявлялися ділянки різних розмірів і форми сіруватого, синюшного та червоного кольору. У деяких випадках печінка була нерівномірно забарвлена (з ділянками жовтуватого та глинистого кольору), а в інших випадках під її капсулою були виявлені точкові та плямисті крововиливи. Однак, найбільш виразні та постійні грубі зміни реєструвались у репродуктивних органах. Під час гістологічних досліджень нирок курей-несучок, інфікованих вірусом СЗН, ми виявили, що в цьому органі є специфічні для даного захворювання та неспецифічні для нього мікроскопічні зміни, які реєструються при ураженні нирок різної етіології. Специфічні для СЗН зміни включали наявність базофільних та еозинофільних тілець-включень у ядрах багатьох епітеліальних клітин звивистих канальців, частини епітеліальних клітин прямих канальців та частини ендотеліальних клітин, подоцитів та мезангіоцитів багатьох клубочків. Деякі ядра повністю набули виразно оксифільних властивостей. Неспецифічні мікроскопічні зміни включали різноманітні зміни ядер та цитоплазми епітеліальних клітин звивистих та прямих канальців, порушення міжклітинних з'єднань, пошкодження ниркових тілець.*

Ключові слова: *кури-несучки, синдром зниження несучості, клінічні ознаки, макроскопічні зміни, нирки, мікроскопічні зміни*

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BIOLOGICAL PROPERTIES OF *STAPHYLOCOCCI* DERIVED FROM CATS AND DOGS

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Abstract. *Dogs and cats that live near humans are a source of zoonotic agents. The purpose of the study was to investigate domestic dogs and cats for staphylococcus carriers and to study their biological properties, biofilm ability, and antibiotic resistance in isolated cultures. A total of 44 samples were collected from 19 dogs and 25 cats of different age and sex groups. Staphylococci were detected in 54% of all samples selected. 25 cultures of Staphylococcus spp. were isolated. Plasma coagulation reaction was negative in 100% of strains isolated from cats and positive in 2 strains isolated from dogs (18%). Lecithinase activity was detected in 85.8% of strains isolated from cats and 72.8% from dogs. 71.5% of strains isolated from cats and 63.7% from dogs had the ability to induce hemolysis. Mannitol was fermented in 50.0% of strains isolated from cats and 54.6% from dogs. 78.6% of strains isolated from cats and 91.0% isolated from dogs grew on crystal violet lactose agar in the*

[1] Scientific Supervisor – Doctor of Veterinary Sciences, Professor V. O. Ushkalov

form of blue colonies. Simultaneously 28.5% of strains isolated from cats and 27.2% isolated from dogs showed the lecithinase activity, hemolysis, fermented mannitol, and grew on crystal violet lactose agar. The derived strains from cats and dogs had multiple resistance to Oxacillin, two or more groups of antibiotics. As a result of the study of biofilm formation, all 100% of isolates obtained from dogs had a high optical density (OD > 1.0) and the ability to form a biofilm. Only 21.4% of strains isolated from cats formed biofilms with a medium optical density (OD > 0.5 < 1.0), and the remaining 78.5% – high optical density (OD > 1.0) and the ability to form a biofilm.

Keywords: *coagulase-positive and coagulase-negative Staphylococci, biofilms, antibiotic resistance*

Introduction

Zoonoses are infectious diseases that spread between animals and humans and can be caused by microorganisms (bacteria, viruses, microscopic fungi) and parasites. They are among the most common diseases in the food chain of transmission (so-called food poisoning) and can also be transmitted to humans either in direct contact with a diseased animal or with a vector / carrier. According to a 2019 report of the European Food Safety Authority and European Center for Disease Prevention and Control (EFSA and ECDC), there are typically about 5 000 outbreaks of food zoonoses per year in the European Union, affecting more than 40 000 people.

In many cases, patients need hospitalization, and deaths are recorded. Together, they pose a major challenge to the health care system and have important implications for international trade.

Analysis of recent researches and publications

Dogs and cats as the main pets occupy a special place in human life. Often, dogs and cats have various diseases caused by *Staphylococci* microorganisms. The main pathogens are *S. aureus* (Weese & Duijkeren, 2010), *S. schleiferi* (Lee et al., 2019) *S. pseudointermedius, S. haemolyticus* (Kizerwetter-Świda et al., 2019), and many other coagulase-negative *Staphylococci*.

The widespread use of antimicrobials in the treatment of humans and their pets has led to the activation of resistance in certain types of microorganisms, in particular, in *Staphylococci*. One of the representatives of *Staphylococci* – the methicillin-resistant (MRSA*) S. aureus*. As a result of an in-depth study of the resistance of *Staphylococci* to antibiotics, a separate group of methicillin resistant (MRS) and methicillin susceptible (MSS) – *S. aureus* LA (livestock animals) was derived. *Staphylococcus* strains containing antibiotic resistance genes colonize the mucous membranes and skin of a wide variety of animal species, especially domestic dogs and cats. Numerous studies (Weese & Duijkeren, 2010; Hatch et al., 2012; Quitocoet al., 2013; Savini et al., 2013; Worthing at al., 2018; Gómez-Sanz et al., 2019; Loncaric et al., 2019) are devoted to the study of the transmission of *Staphylococci* from dogs and cats to humans and their biological properties. *Staphylococci* derived from dogs and cats are known to have a different set of pathogenicity features, such as the presence of coagulase lecithinase, hemolytic activity, toxin forma-

tion, biofilm formation, etc. (Rahman et al., 2018; Loncaric et al., 2019). At present, the phenomenon of biofilm formation needs to be thoroughly studied. It is known that they are formed due to the action of aggressive factors: detergents, disinfectants, antibiotics, and probiotics (Paharik & Horswill, 2016; Ahmadrajabi et al., 2017; Stewart et al., 2017; Otto, 2018; Turko & Ushkalov, 2018; Vitale et al., 2019). In 2017, 80 MRS strains were isolated in Ukraine, including 77.5% from domestic animals, 11.3% from poultry, 6.3% from cattle, and 5% from pigs (Kozytska et al., 2019).

Purpose. In view of the above, the aim of our work was to investigate domestic dogs and cats as the *Staphylococcus* carriers and to study the biological properties of the selected cultures, in particular, the ability to biofilm and antibiotic resistance.

Materials and methods of research

Work was carried out in the Research Department of Microbiological Research of the Ukrainian Laboratory for Quality and Product Safety of the Agro-industrial Complex of the National University of Life and Environmental Sciences of Ukraine during August–March 2019–2020. The clinical research material was selected at the "Multivet" veterinary clinic in the Kyiv region. A total of 44 samples were collected from 19 dogs and 25 cats of different age and sex groups (Table 1).

Selection and identification of *Staphylococcus spp*. was carried out in accordance with DSTU EN 6888: 2003 "Microbiology of food products and animal feed". Using the Baird-Parker agar medium (Merck, Germany). In the derived cultures, colony morphology, lecithinase activity, Gram staining, and catalase test were performed. Hemolytic properties were studied on Colombian blood agar (Biomeriux, France) at a temperature of 37 °C for 24 hours. The coagulase activity *in vitro* by lyophilized citrate plasma rabbit (Biolik, Ukraine) was determined. The formed clots were evaluated visually after 2, 4, 18, and 24 hours of incubation at a temperature of 37 °C. The main characteristics of the colonies growth on Mannitol Salt agar (Merck, Germany) were determined. The sowing was performed by looping on the agar surface, cultured at temperature of 37 °C for 48 hours, and the colonies were evaluated visually. The presence of colonies on Crystal Violet Lactose agar (HiMedia, India) and their color were assessed visually.

Antibiotic resistance of cultures was determined by the disc diffusion method (Kirby-Bauer) using antibiotic discs and a Müller-Hinton agar medium (HiMedia, India), McFarland inoculum 0.5 optical density was determined using a DEN-1 densitometer (Biosan, Latvia) and McFarland standard (HiMedia, India). The incubation was carried out at a temperature of 37 °C for 18 ± 2 hours. The resistance of the obtained strains to Benzylpenicillin,

1. Quantitative characterization of selected samples by disease groups

Type	Number of samples	Pathologies				
		Skin	Eyes	Ears	Traumas	Clinically healthy
Dogs	19	2	4	2	5	6
Cats	25	3	5	3	2	12

Ampicillin, Oxacillin, Norfloxacin, Ciprofloxacin, Levofloxacin, Gentamicin, Tobramycin, Erythromycin, Tetracycline, Doxycycline, and Chloramphenicol was investigated. The results were evaluated in accordance with the recommendations of the European Committee for Antimicrobial Sensitivity EUCAST (version 10.0) and the national criteria for the assessment of antibiotic resistance – the methodological guidance "Determination of susceptibility of microorganisms to antibacterial drugs".

The ability to form biofilms in the derived isolates was determined and the results obtained were interpreted (Kukhtyn & Krushelnytska, 2014). This study was performed using sterile polystyrene Petri dishes (Greiner Bio-One GmbH, Germany) of d = 35 mm, which were added to 5 mL of Tryptone-soy broth (HiMedia, India) and added 1 ml of inoculum with a cell content of 0.5 to MacFarland daily culture of studied *Staphylococci*. The plates were cultured in a thermostat at a temperature of 37 °C for 24 hours, the residues of the nutrient medium were carefully removed, the planktonic forms were washed three times with a sterile phosphate buffer solution (KH_2PO_4 x Na_2PO_4 x H_2O) (pH 7.2–7.4). The Petri dishes were air-dried and 5 mL of Ethanol 96 % was added to fix the formed biofilms. The fixation exposure was 10 minutes. Then the fixing liquid was drained and after it, the Petri dishes were stained with 0.1% alcohol solution of crystal violet for 10 min. Then washed the plates three times with sterile phosphate buffer solution (pH 7.2) and dried. Contributed to 5 mL of Ethanol 96% and placed on a shaker for shaking for 30 min. The contents of the Petri dishes were then pipetted and the amount of absorption of the biofilm of the dye was measured on an Evolution 300 spectrophotometer (Thermo Fisher Scientific, USA) at a wavelength of 570 nm. The density of the formed biofilm was determined by measuring the adsorption level of the dye with ethanol measured in units of optical density (OD) using a spectrophotometer.

When the value of an optical density is less than 0.1, it was considered that the strains do not form a biofilm, from 0.1 to 0.49 – the ability to form a film was considered as a low. When the value of optical density is from 0.5 to 1.0 – the medium density of the biofilm and the ability to form it. At values above 1.0 – the high ability to form a biofilm and its high density (Kukhtyn & Krushelnytska, 2014).

Results of the research and their discussion

According to the results of the experiments, *Staphylococci* were detected in 54% of all samples. Total of 25 *Staphylococcus spp.* cultures were derived and investigated.

On the Baird-Parker agar, isolated cultures grew in the form of typical and not typical colonies. Typical colonies are black and gray, shiny, and convex of 1.0–2.5 mm in diameter, surrounded by a clear area. The atypical colonies were brilliant black colonies with a narrow white margin and gray colonies, with no clear area. Gram staining revealed gram-positive spherical bacteria, which occurred in the form of clusters that resemble grapes, fixed, did not form a spore. They gave a positive catalase reaction.

Plasma coagulation was negative in 100% of cat isolates and positive in 2 dogs isolates (18 %). Lecithinase activity was shown by 85.8% of the strains isolated from cats and 72.8% – from dogs (Fig. 1).

71.5% of strains isolated from cats and 63.7 % from dog possessed the hemolysis ability. Mannitol was fermented with 50.0% of the strains isolated from cats and 54.6% from dogs (Fig. 2, 3).

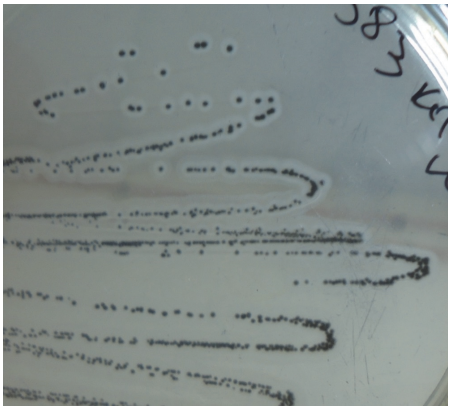

Fig. 1. Lecithinase activity on Baird-Parker agar (HiMedia, India)

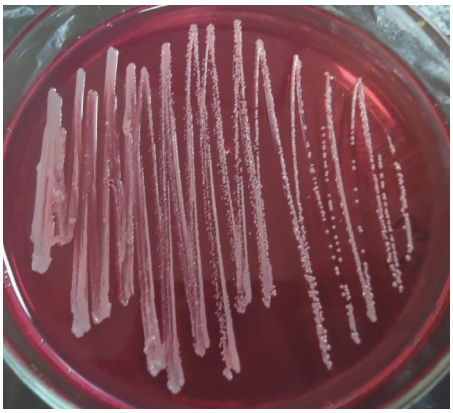

Fig. 3. No fermentation of mannitol on Mannitol Salt agar (Merck, Germany)

On crystal violet lactose agar, 78.6% of the strains isolated from cats and 91.0% isolated from dogs were formed in blue colonies (Fig. 4). At the same time, the lecithinase activity, hemolysis, fermented mannitol and grew on crystal violet lactose agar exhibited 28.5% of strains isolated from cats and 27.2% isolated from dogs (Fig. 5).

28.5% of strains derived from cats and 54.5% of strains derived from dogs were resistant to Benzylpenicillin (Fig. 6). 28.5% of strains derived from cats and 27.2% of strains derived from dogs were resistant to Ampicillin. 28.5% of strains derived from cats and 45.4% of strains derived from dogs were resistant to Oxacillin (Fig. 7).

14.2% of strains derived from cats were resistant to Norfloxacin and Ciprofloxacin.

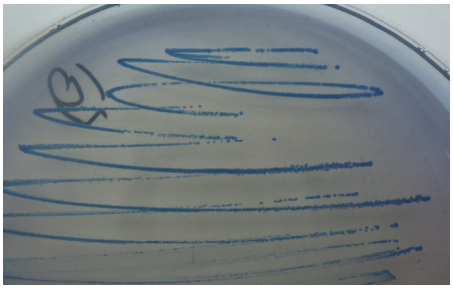

Fig. 4. Blue colonies on crystal violet lactose agar (HiMedia, India)

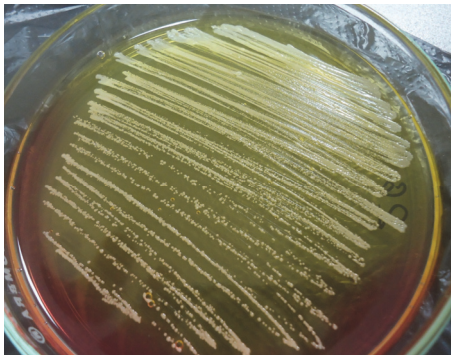

Fig. 2. Mannitol fermentation on Mannitol Salt agar (Merck, Germany)

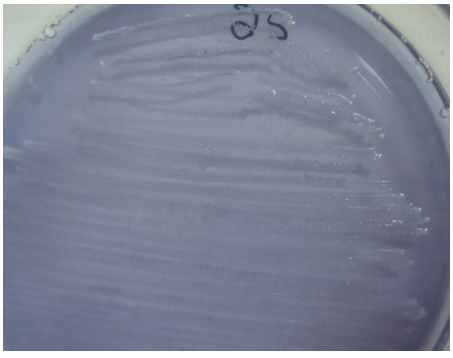

Fig. 5. White colonies on crystal violet lactose agar (HiMedia, India)

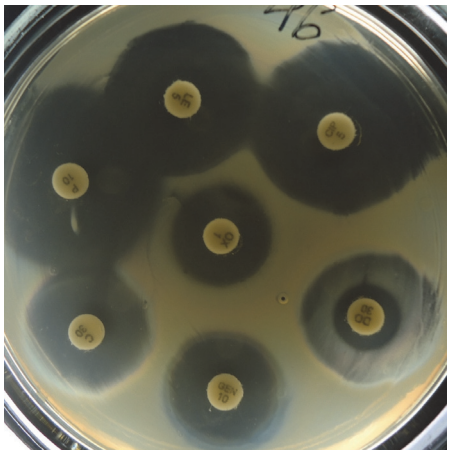

Fig. 6. Susceptible to Oxacillin and Benzylpenicillin strains of *Staphylococcus spp.*

27.2% of strains derived from dogs were resistant to Norfloxacin and Ciprofloxacin. 7.1% of strains derived from cats and 27.2% of strains derived from dogs were resistant to Levofloxacin. 21.4% of strains derived from cats and 27.2% of strains derived from dogs were resistant to Gentamicin and Tobramycin. 42.8% of strains derived from cats and 27.2% of strains derived from dogs were resistant to Erythromycin. 28.5% of strains derived from cats were resistant to

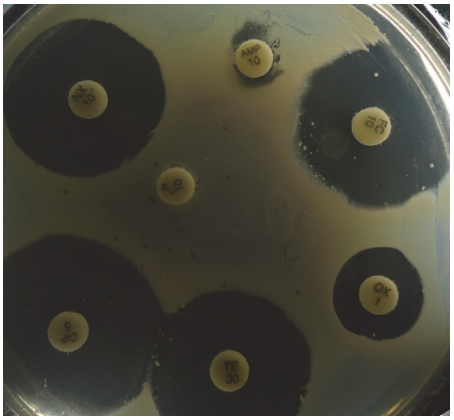

Fig. 7. Oxacillin, Ampicillin, and Benzylpenicillin resistant strains of *Staphylococcus spp.*

Tetracycline. 27.2% of strains derived from dogs were resistant and 9.0% were moderately resistant to Tetracycline. 14.2% of strains derived from cats and were intermediately resistant to Doxycycline. 18.1% of strains derived from dogs were resistant to Doxycycline. 14.2% of strains derived from cats and 36.3% of strains derived from dogs were Chloramphenicol-resistant. 21.4% of strains derived from cats and 36.3% of strains derived from dogs were resistant to Fusidic acid. The isolated strains from cats and dogs had multiple resistances to two or more antibiotic groups. Thus, a coagulase-negative strain resistant to all antibiotics except Levofloxacin, Fusidic, acid and moderately resistant to Doxycyclin was derived from cats. Two resistant strains to the Penicillin group, Erythromycin, and Chloramphenicol, were also derived. A strain resistant to all antibiotic groups, except for Chloramphenicol and Fluoroquinolones, was also derived from cats. 35.7% of strains derived from cats were sensitive to all studied antibiotic groups.

Two coagulase-positive strains isolated from dogs exhibited different resistance. Thus, one strain was sensitive to Benzylpenicillin, Ampicillin, Erythromycin, Oxacillin, and, at the same time, was resistant to all other antibiotics. Another coagulase-positive strain was resistant to Benzylpenicillin, Gentamicin, Tobramycin, and Fusidic acid, and, at the same time, was sensitive to other antibiotics. Among the coagulase-negative strains isolated from dogs, one strain was resistant to all antibiotics, except for Doxycycline; one strain was resistant to all antibiotics, except for Ampicillin, Gentamicin, and Tobramycin. One strain resistant to the Penicillin and Erythromycin group and two strains resistant to the Penicillin group were also isolated. 27.2% of strains were sensitive to all groups of antibiotics tested.

2. The value of the optical density of biofilms at 570 nm and the ability to form biofilms in strains of Staphylococci isolated from cats and dogs (M ± m, n = 3)

Strain No.	The value of the optical density of the strains derived from cats	The value of the optical density of the strains derived from dogs
1	3.184 ± 0.095	4.744 ± 0.188
2	0.909 ± 0.030	3.440 ± 0.134
3	1.651 ± 0.060	1.896 ± 0.065
4	1.243 ± 0.037	2.138 ± 0.088
5	0.908 ± 0.033	2.483 ± 0.052

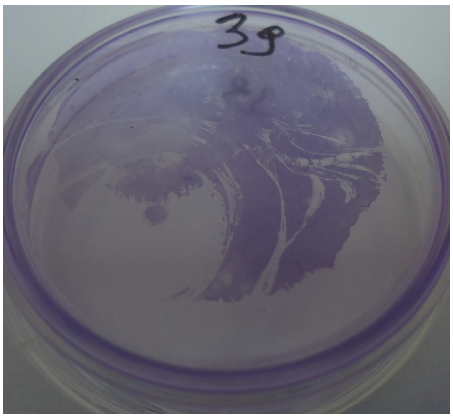

Fig. 10. The high density of the formed biofilm

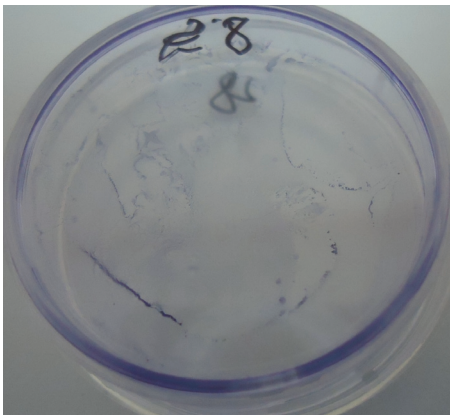

Fig. 11. The medium density of the formed biofilm

As a result of the study of biofilm formation, all 100% of the isolates isolated from dogs had a high optical density (OD > 1.0) and the ability to form a biofilm (Table 2).

Only 21.4% of strains isolated from cats formed biofilms with medium optical density (OD > 0.5 <1.0), and the remaining 78.5% – with high optical density (OD > 1.0) and the ability to form a biofilm (Fig. 10).

Conclusion and future perspectives

Staphylococcus spp. was derived from 54.0% of studied animals. Selected cultures have different pathogenicity factors (lecithinase, coagulase). Simultaneously showed lecithinase activity, hemolysis, fermented mannitol, and grew on crystal violet lactose agar 28.5% of strains isolated from cats and 27.2% isolated from dogs. The studied cultures of *Staphylococcus spp.,* had multiple resistance to Oxacillin, two or more groups of antibiotics. As a result of the study of biofilm formation, all 100% of isolates obtained from dogs had a high optical density (OD > 1.0) and the ability to form a biofilm. Only 21.4% of strains isolated from cats formed biofilms with a medium optical density (OD > 0.5 <1.0), and the remaining 78.5% – high optical density (OD > 1.0) and the ability to form a biofilm. The data obtained by us indicate the need for full monitoring of antibiotic re-

sistance and biofilm capacity of *Staphylococci* isolated from domestic animals using polymerase chain reaction analysis.

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Анотація. *Собаки й коти, які мешкають поруч із людьми, є джерелом збудників зоонозів. Метою роботи було дослідити домашніх собак і котів на носійство стафілококів і вивчити їхні біологічні властивості, здатність до біоплівкоутворення та стійкість до антибіотиків у виділених культур. Усього було відібрано 44 зразки від 19 собак та 25 котів різних вікових та статевих груп. У 54% з усіх відібраних зразків були виявлені стафілококи. Ізольовано 25 культур Staphylococcus spp. Реакція плазмокоагуляції була негативною у 100% штамів отриманих від котів і позитивною – у 2 штамів отриманих від собак (18%). Лецитиназну активність проявляли 85,8% штамів ізольованих від котів і 72,8% – від собак. Здатністю до гемолізу володіли 71,5% штамів виділених від котів і 63,7% – від собак. Ферментували манітол 50,0 % ізолятів від котів і 54,6% – від собак. Росли на лактозному агарі з кристалічним фіолетовим у вигляді синіх колоній 78,6% штамів отриманих від котів і 91,0% – від собак. Одночасно проявляли лецитиназну активність, гемоліз, ферментували манітол і росли на лактозному агарі з кристалічним фіолетовим 28,5 % штамів ізольованих від котів і 27,2% – від собак. Отримані штами від котів і собак володіли множинною резистентністю до оксациліну, двох і більше груп антибіотиків. Результати дослідження утворення біоплівок показали, що усі 100 % ізолятів виділених від собак володіли високою оптичною щільністю (OD > 1.0) та здатністю до формування біоплівки. Лише 21,4% штамів ізольованих від котів утворювали біоплівки середньої оптичної щільності (OD > 0.5 < 1.0), а решта 78,5% – високої оптичної щільності (OD > 1.0) та здатності до утворення біоплівки.*

Ключові слова: *коагулазопозитивні і коагулазонегативні Staphylococci, біоплівки, антибіотикорезистентність*

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VETERINARY SCIENCE AND ANIMAL HUSBANDRY TOPOGRAPHY AND MORPHOLOGY OF THORACO-CERVICAL LYMPH NODES OF DOMESTIC GEESE

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Abstract. *It is established that in geese, are constantly registered only cervicothoracic lymph nodes. The cervicothoracic lymph nodes of geese are local accumulations of lymphoid tissue in the wall of the lymphatic vessels, which are taking away lymph from the head and neck. The cervicothoracic lymph nodes of geese are not the same shape, most of them have a conical shape. Parameters of absolute, relative weights, and size of the cervicothoracic lymph nodes of geese depend on the weight and sizes of their body.*

The cervicothoracic lymph nodes of geese are formed by a connective tissue stroma, parenchyma, and sinus. Connective tissue stroma is represented by a capsule, which is a continuation of the adventitia and media of lymphatic vessels and trabeculae. Connective tissue stroma is formed by loose fibrous connective tissue, which has blood vessels and myocytes. Sinus is a continuation of the lymphatic vessel lumen and its wall – a continuation of its intima. The area, occupied by a connective tissue stroma, parenchyma, and sinus in the nodes of investigated birds, varies. The largest area occupies the parenchyma of the cervicothoracic lymph nodes, which like the parenchyma of lymph nodes in mammals, is formed by lymphoid tissue. In the parenchyma, there is unclearly allocated the cortical and cerebral substances. The cortical substance is located on the periphery and the cerebral substance – around the sinus. The area of the cortical substance dominates over a cerebral substance. The cortical substance is formed by the diffuse lymphoid tissue and lymphoid nodules (mainly secondary), and the cerebral substance – only by the diffuse lymphoid tissue. Lymphoid nodules are spherical in shape, surrounded by delicate shells, and depending on the diameter, is divided into three groups: large, medium, and small. The basis of the lymphoid tissues in the parenchyma is reticular tissues, which consists of reticular fibers. The architectonics of its fibers varies in the different components of the lymphoid tissue. They are placed tightly, intertwined, and form the confined units of various shapes and small sizes. The cellular composition of the parenchyma of the cervicothoracic lymph nodes of geese is extremely diverse. It is reticulocytes, lymphocytes, immunoblasts, macrophages,

monocytes, plasma cells, mast cells, and myoid cells. The content of these cells is not the same. The presence of lymphocytes, immunoblasts, macrophages, and plasma cells in the parenchyma of the cervicothoracic lymph nodes of geese convinces in their functioning as peripheral organs of immunogenesis.

Keywords: *geese, cervicothoracic lymph nodes, connective tissue stroma, parenchyma, central sinus, cortical substance, medullary substance, lymphoid follicles, lymphoid cells*

Introduction

Lymph nodes are the peripheral organs of haematopoiesis and immunogenesis (Sapin et al., 1978; Vershigora, 1990). In them, due to antigenic stimulation, T- and B-lymphocytes differentiate into the effector cells, which cause specific immunity. Besides, in these bodies, there is a release of lymph from the foreign substances and its deposition, recirculation of lymphocytes, an metabolism of some substances. They, owing to availability in their connective tissue stroma of smooth myocytes, also promote lymph circulation (Vinogradova, 1970; Sapin, 1977; Sapin, 1978; Sapin et al., 1978; Vershigora, 1990).

Analysis of recent researches and publications

During the historical development of animals for the first time lymph nodes appear in waterfowl (Sapin et al., 1978). They are found in geese, ducks, flamingos, and are presented by two couples: the cervicothoracic and lumbar (Jolly, 1909–1910; Ellenbergez & Baum, 1915; Kositsyn, 1958; Kositsyn, 1959; Kositsyn, 1963; Florensov, 1968; Florensov & Pestova, 1990). References about the existence of lumbar lymph nodes at waterfowls are inconsistent. There are data that geese and ducks don't have them and that they are assimilated with kidneys (Pylypenko & Musienko, 1973; Berezina, 1979).

The topography and morphology of the cervicothoracic lymph nodes of birds are studied insufficiently. Due to this, the data of their functional features are incomplete and contradictory.

The purpose of the study – to clarify the topography of the cervicothoracic lymph nodes of geese at the age of puberty, to establish their macroscopic morphological parameters, and to find out features of the microscopic structure.

Materials and methods of research

Material for studying was selected from 13 geese of Gorki Goose breed at the age of 11 months which grew up in farms of Kyiv region (Ukraine). To perform the work, the classical methods of macroscopic and microscopic morphological studies (Merkulov, 1969; Avtandilov, 1990; Goralsky et al., 2005) were used. After the slaughtering of birds by an acute devascularization, the topography, shape, color, texture, absolute and relative organ weight, and linear morphometric parameters were defined. For the investigation of the cellular composition of lymph node parenchyma, the imprint preparations were prepared. The materials selected for microscopic examination were fixed in a 10% aqueous solution of neutral formalin for 5 days. From the fixed material were cut indi-

vidual pieces, which were dehydrated in ethyl alcohol with the increasing strength and embedded in paraffin. The obtained paraffin blocks were sliced by microtome MPS-2 with a thickness of 3–8 microns. Slices were stained with hematoxylin and eosin, and by Weigert and van Gizon. The composition of parenchyma cells, their structure, and content were determined by histological preparations stained with azure II and eosin, imprint preparations stained according to Pappenheim, and by electron microscopy according to the V. Uikly method (Goralsky et al., 2005).

Bright-field microscopes "Olympus", "Biolar", MBI-15 MBI-6-1, and MBS-1 were used for microscopic studies. The area to be occupied in the lymph nodes of individual structural components were determined using a microscope MBS-1 and the measuring grid, which is a part of its set.

Results of the research and their discussion

The performed researches confirmed the data (Khomych & Melnyk, 2008; Melnyk, 2010), that the cervicothoracic lymph nodes of geese are paired constant bodies. They are located under the skin in a lower part of the neck, at the top of the angle which is formed by the jugular and subclavian veins (before the entry of a jugular vein into a thoracoabdominal cavity) (Fig. 1). Lymph nodes are covered with many layers of fatty tissue that complicate their detection, preparation, and differentiation from a number of the located last lobes of the thymus.

The cervicothoracic lymph nodes have different forms. They may be cone-shaped (80%) and spindle-shaped (20%). Their colours are pink and grey, and consistency is dense.

The absolute weight of the right and left lymph nodes is the same (0.28 ± 0.06 g), and the relative weight of the first ($0.009 \pm 0.001\%$) is bigger than the second ($0.008 \pm 0.001\%$).

The length of the right lymph node (25.68 ± 2.20 mm) is bigger than the left (22.85 ± 1.56 mm). Width in the area of expansion: the right – 4.65 ± 0.47 mm, the left – 5.46 ± 0.59 mm. The width of the restriction: the right – 2.69 ± 0.38 mm, the left 3.34 ± 0.49 mm. The thickness of the lymph nodes: the right – 2.038 ± 0.242 mm, the left – 2.331 ± 0.345 mm.

The cervicothoracic lymph nodes of geese located in a wall of lymphatic vessels, which are taking away lymph from the head and the neck, and in a formation of their components, except for lymphoid tissue, participate all structures of these vessels. They are formed by a connective tissue stroma and parenchyma of the central sinus. Connective tissue stroma occupies $23.95 \pm 0.95\%$ of the area of nodes, parenchyma – $55.48 \pm 1.45\%$, and the sinus – $20.57 \pm 0.66\%$.

Connective tissue stroma is presented by a capsule and a few trabeculae (Fig. 2). The capsule is a continuation of adventitia and media of a lymphatic vessel. In comparison with them, it is thickened. Trabeculae extend from the capsule into the parenchyma of the nodes. They do not have a specific orientation and do not reach the walls of the sinus. Connective tissue stroma is formed by loose fibrous connective tissue with its characteristic cells and intercellular substance. The stroma contains bundles of single bunches of myocytes and blood vessels.

Sinus occupies a central position in the lymph nodes. It is a continuation of the lumen of the lymphatic vessel and its wall – a continuation of its intima (Fig. 3).

The wall of the sinus is formed by a layer of endothelial cells and a subendothe-

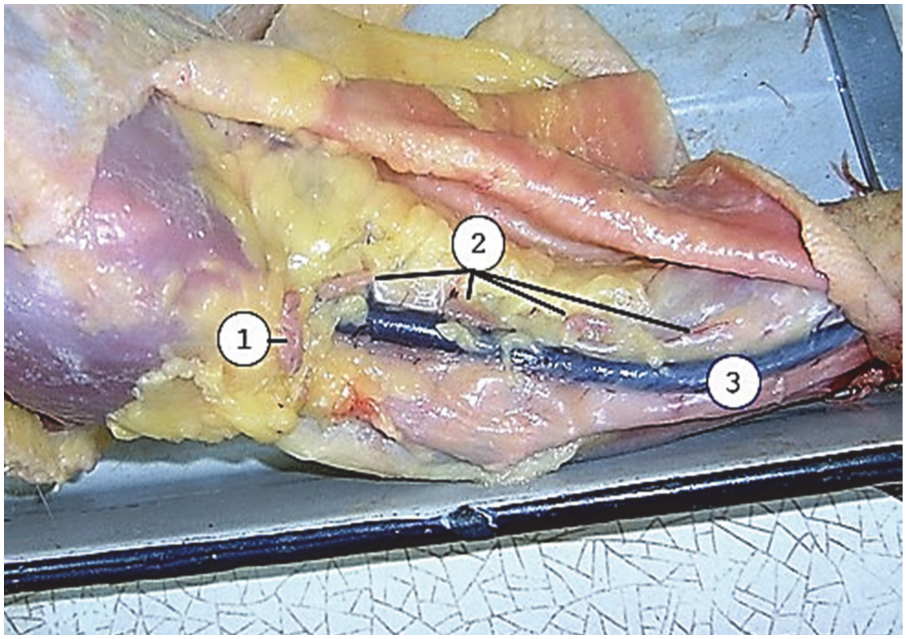

Fig. 1. Topography of the cervicothoracic lymph nodes of a domestic goose. Macropreparations: 1 – lymph node; 2 – thymus lobes; 3 – jugular vein

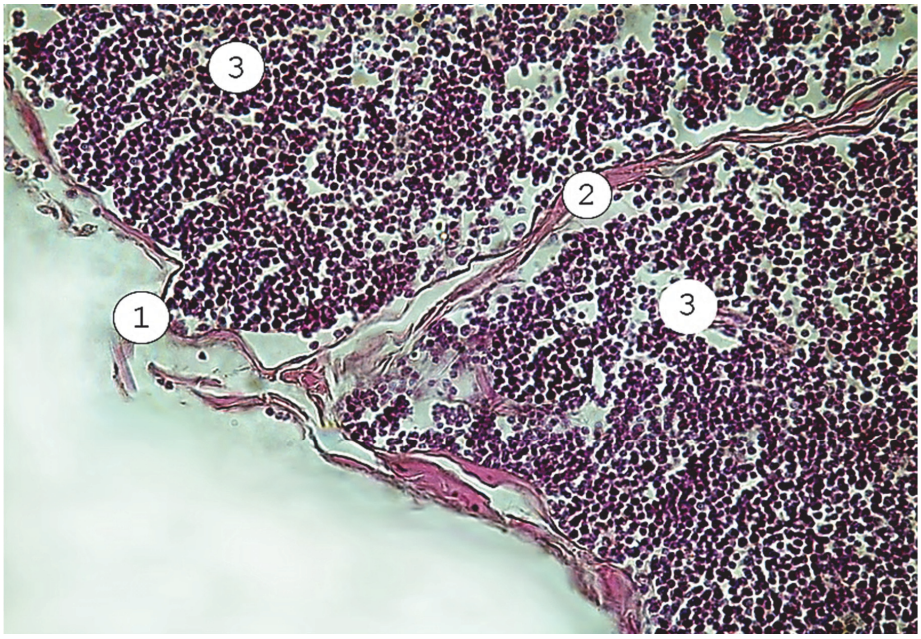

Fig. 2. Connective tissue stroma of the cervicothoracic lymph node of a domestic goose. Histological preparation (hematoxylin and eosin, x 200): 1 – capsule; 2 – trabeculae; 3 – parenchyma

lial layer. The structure of these endothelial cells is similar to the cells of lymphatic vessels in mammals. In some places between the endothelial cells, there are significant cracks through which there is a migration of lymphoid cells into the lumen of the sinus. We didn't find the basal membrane under endothelial cells. The subendothelial sinus wall layer is formed by a soft layer of a loose fibrous connective tissue. Among its fibers, we found the only collagen, which in places, are connecting with the outer surface of the endothelial cells. Axons of the reticular cells of a node's parenchyma penetrate the subendothelial layers. Sinus varies in width. In some places its' wall penetrates a node's parenchyma, forming deepings of various shapes and sizes.

In a sinus lumen, there are fragments of disrupted cells, surrounded by a membrane, lymphoid cells, and single erythrocytes.

The parenchyma of lymph nodes is located between a wall of a sinus and components of connective tissue stroma. Its basis is formed by lymphoid tissue. In the parenchyma, there is unclearly stand out the cortical and cerebral substances. The first is located on the periphery, the second – around the sinus and its invaginations. The area of the cortical substance (57.99 ± 1.21%) dominates under a cerebral substance (42.01 ± 1.21%). The cortical substance is formed by the diffuse lymphoid tissue and lymphoid nodules (mainly secondary), and the cerebral substance – only by the diffuse lymphoid tissue (Fig. 4). Lymphoid nodules are predominantly spherical in shape, have an unequal diameter, and are surrounded by delicate shells. Depending on the diameter, we divided them into a small (58.79 ± 1.37 mm), medium (165.51 ± 1.93 mm), and large (253.16 ± 3.43 mm).

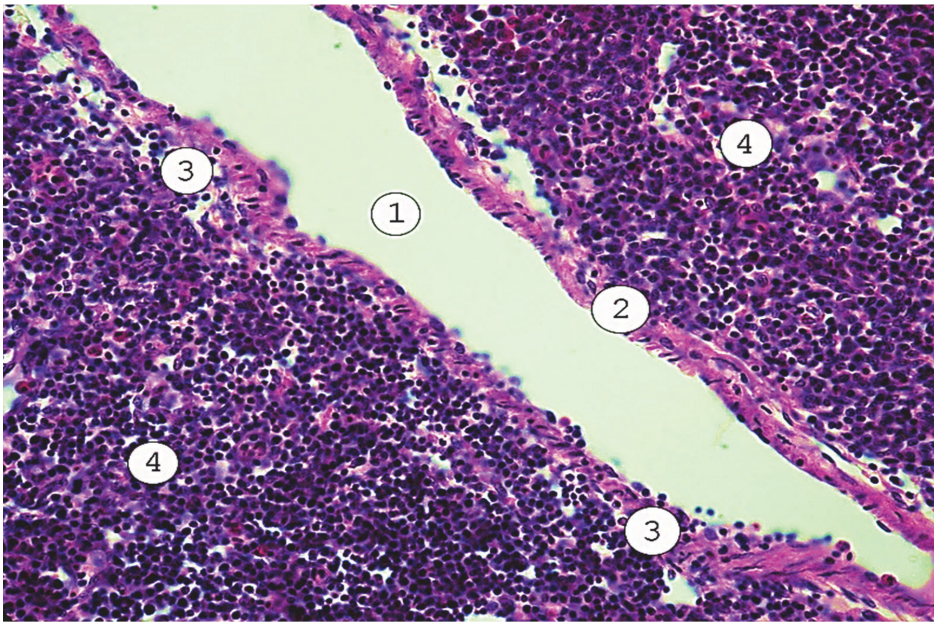

Fig. 3. Central the cervicothoracic sinus node of a domestic goose. Histological preparation (hematoxylin and eosin, × 200): 1 – lumen of the sinus; 2 – wall of the sinus; 3 – invaginations; 4 – node parenchyma

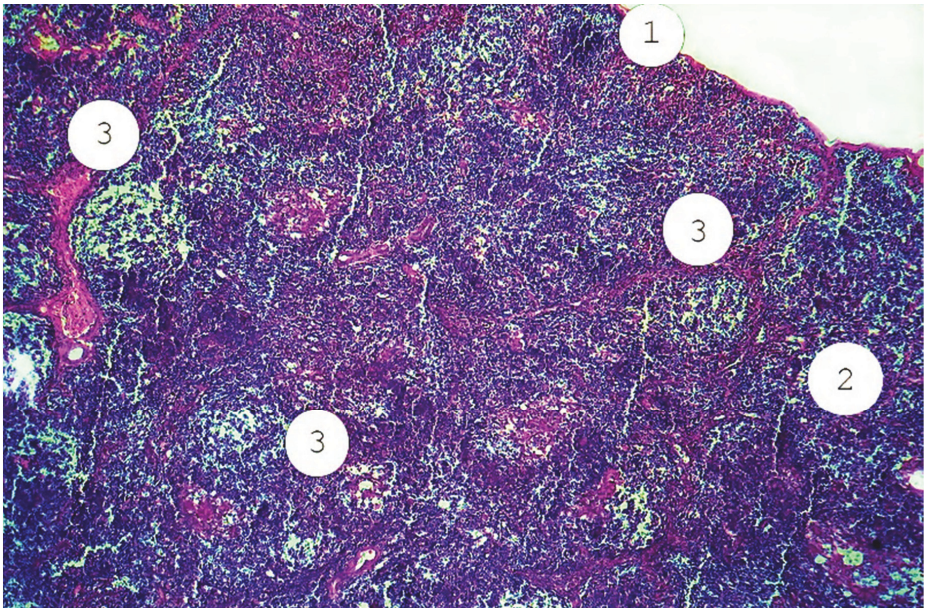

Fig. 4. Cortical substance of the cervicothoracic lymph node of a domestic goose. Histological preparation (hematoxylin and eosin, x 100): 1 – capsule; 2 – diffuse lymphoid tissue; 3 – lymphoid nodules

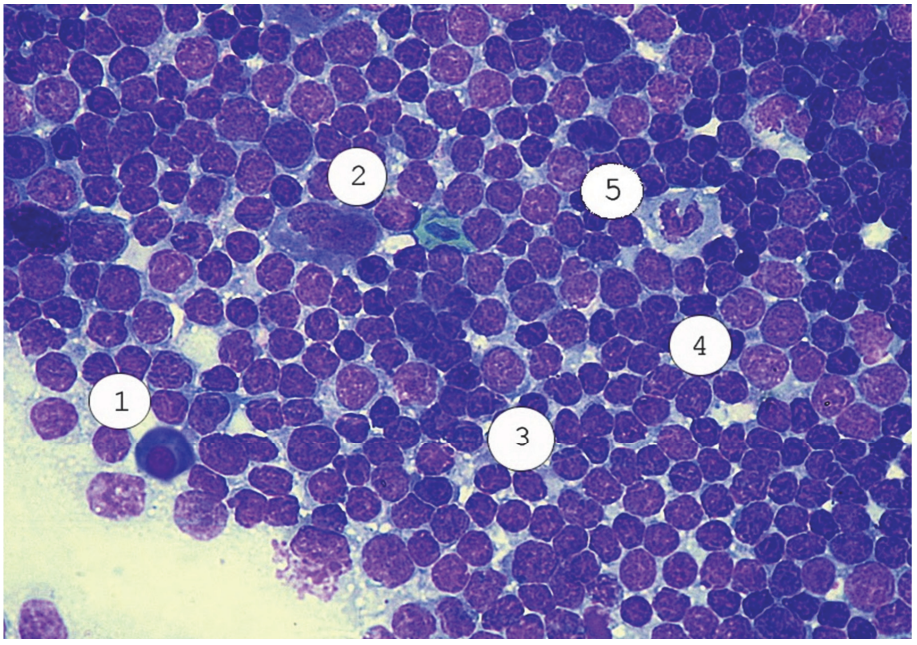

Fig. 5. Parenchyma cells of lymph node of a domestic goose. The preparations (imprint stained by Pappenheim, × 1000): 1 – plasmocytes; 2 – macrophage; 3 – large lymphocytes; 4 – medium lymphocytes; 5 – monocyte

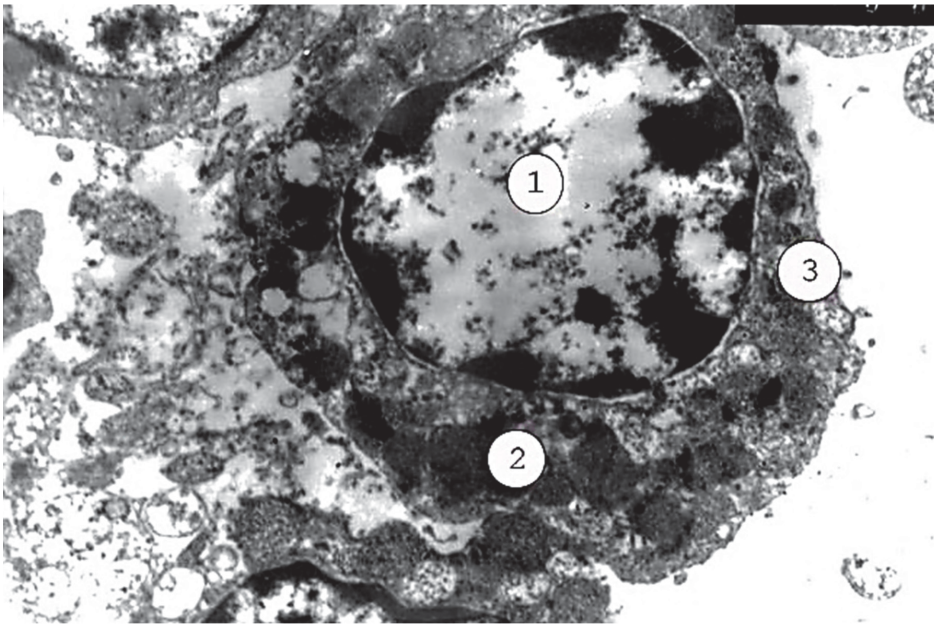

Fig. 6. Mast cell in parenchyma of the cervicothoracic lymph node of a domestic goose. Electronogram, × 13500: 1 – nucleus; 2 – granules in cell cytoplasm; 3 – cell cytoplasm

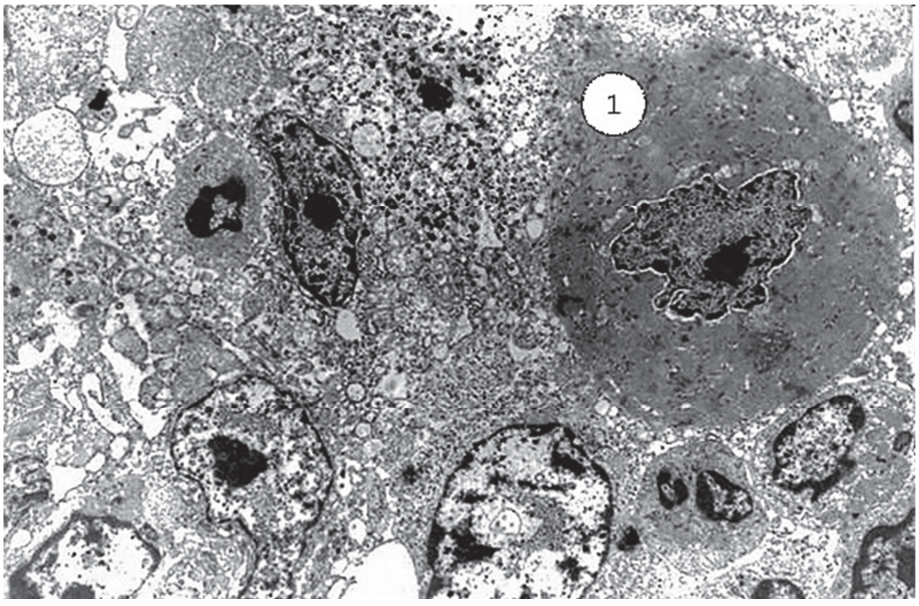

Fig. 7. Myoid cell (1) in parenchyma of the cervicothoracic lymph node of a domestic goose. Electronogram, × 6500

The basis of the lymphoid tissue of parenchyma node is formed by a reticular tissue. The architectonics of its fibers varies in the varieties of lymphoid tissue. They form a fine mesh in the diffuse lymphoid tissue, cortex, and cerebral substance. In grid cells of lymphoid nodules, larger reticular fibers are often visible, and their center can be absent.

In imprint preparations of the cervicothoracic lymph nodes, in their parenchyma were found reticulocytes, lymphocytes, immunoblasts, plasma cells, macrophages, monocytes, mast cells, and myoid cells (Fig. 5–7).

The established cellular structure of parenchyma in studied lymph nodes testifies that these geese organs function as peripheral organs of immunogenesis.

The content of the parenchyma cells in the lymph nodes is not the same.

Among them, there are more lymphocytes (79.51 ± 2.73%). Among the lymphocytes, there are large, medium, and small.

The second place by cell content immunoblasts occupy (18.73 ± 1.05%).

The contents of plasmacytes, macrophages and their predecessors – monocytes is insignificant (0.64 ± 0.07% and 1.12 ± 0.06%).

The presence of lymphocytes, immunoblasts, macrophages, and plasma cells in parenchyma of the cervicothoracic lymph nodes of geese indicates that they function as peripheral organs of immunogenesis.

Conclusions

In mature domestic geese are constantly registered paired cervicothoracic lymph nodes and lumbar are absent. The cervicothoracic lymph nodes are predominantly spindle-shaped, less – conical. Their absolute and relative weights and sizes also depend on the weight and the size of the body of birds. The absolute weight of the left cervicothoracic lymph node of geese is 0.28 ± 0.06 g, the right – 0.28 ± 0.06 g. The relative weight of the right cervicothoracic lymph node of geese is 0.009 ± 0.001%, the left – 0.008 ± 0.001%.

The cervicothoracic lymph nodes of domestic geese are formed by a connective tissue stroma, parenchyma, and the central sinus. The cortical substance of parenchyma is located on the periphery and the cerebral substance – surrounds the sinus. The first is formed by lymphoid nodules and diffuse lymphoid tissue, and the second – only by the diffuse lymphoid tissue.

As a part of the parenchyma of the cervicothoracic lymph nodes, except for the cells, which contribute the function as a peripheral organ of immunogenesis (reticulocytes, immunoblasts, lymphocytes, plasmocytes, monocytes, and macrophages), mast cells and myoid cells are also identified. There are many lymphocytes among them (79.51 ± 2.73%).

The architectonics of reticular fibers in the parenchyma of the cervicothoracic lymph nodes varies in its individual components. In the diffuse lymphoid tissue of the lymph nodes, they form a fine mesh, on a periphery of lymphoid nodules of the reticular fibers there is a coarse mesh, and in their center – fibers are located solitary.

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Анотація. *Встановлено, що в гусей постійно реєструються тільки грудо-шийні лімфатичні вузли. Грудо-шийні лімфатичні вузли гусей є локальними скупченнями лімфоїдної тканини в стінці лімфатичних судин, що відводять лімфу від голови й шиї. Форма грудо-шийних лімфатичних вузлів у гусей неоднакова, більшість із них мають конусоподібну форму. Показники абсолютної, відносної маси і розмірів грудо-шийних лімфовузлів гусей залежать від маси й розмірів їхнього тіла. Грудо-шийні лімфовузли гусей утворені сполучнотканинною стромою, паренхімою й синусом. Сполучнотканинна строма представлена капсулою, яка є продовженням адвентиції й медії лімфатичної судини, а також трабекулами. Строма складається з пухкої волокнистої сполучної тканини, у якій розміщені міоцити і кровоносні судини. Синус є продовженням просвіту лімфатичної судини, а його стінка – продовженням її інтими. Площа, яку займають сполучнотканинна строма, паренхіма й синус у вузлах досліджених птахів, неоднакова. Найбільшу площу займає паренхіма грудо-шийних лімфовузлів, яка, як і паренхіма лімфовузлів ссавців, утворена лімфоїдною тканиною. У паренхімі нечітко виділяються кіркова і мозкова речовини. Її кіркова речовина розташована на периферії, а мозкова – навколо синуса. Площа кіркової речовини переважає над мозковою. Кіркова речовина утворена дифузійною лімфоїдною тканиною і лімфоїдними вузликами (переважно вторинними), а мозкова – тільки дифузійною лімфоїдною тканиною. Лімфоїдні вузлики круглої форми, оточені оболонками й залежно від діаметра розділені на три групи: великі, середні та малі. Основою лімфоїдної тканини паренхіми є ретикулярна тканина, яка складається з ретикулярних волокон. Архітектоніка волокон неоднакова в різних компонентах лімфоїдної тканини. Вони розташовані щільно, переплітаються і формують замкнуті осередки різної форми й невеликих розмірів. Клітинний склад паренхіми грудо-шийних лімфатичних вузлів гусей надзвичайно різноманітний. Це – ретикулоцити, лімфоцити, лімфобласти (імунобласти), макрофаги, моноцити, плазмоцити, тучні та міоїдні клітини. Склад цих клітин неоднаковий. Наявність у паренхімі грудо-шийних лімфатичних вузлів гусей лімфоцитів, лімфобластів (імунобластів), макрофагів і плазмоцитів вказує на те, що вони функціонують як периферичні органи імуногенезу.*

Ключові слова: *гуси, шийно-грудні лімфатичні вузли, сполучнотканинна строма, паренхіма, центральний синус, кіркова речовина, мозкова речовина, лімфоїдні фолікули, лімфоїдні клітини*

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IXODES TICKS – TRANSMITTERS OF DANGEROUS TRANSMISSIBLE INFECTIONS AND INVASIONS AGENTS

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Abstract. *The article considers domestic and foreign experience in the spread of Ixodes ticks – vectors of dangerous transmissible infections and invasions in the park areas of Ukraine and preventive measures against Ixodidosis of animals and humans. The material for the study was the data of veterinary reporting obtained from the state regional laboratories of the State Service of Ukraine on Food Safety and Consumer Protection for 2018–2019. The research was conducted at the Department of Parasitology and Tropical Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine. Ixodes ticks, previously collected in the park zone of Kyiv, were used for research. We analyzed the results of studying the Ixodes ticks for the presence of pathogens of dangerous transmissible infections and invasions, as well as the treatment of dogs with Ixodidoses.*

We consider it important to timely prevent animals from ticks, the studying of ticks in the areas of their possible spread; analysis of the ecological situation within three to four years in the area where ticks are most common; creation of maps using the Geographic

information systems that reflect the dynamics of diseases transmitted by Ixodes ticks; informing the population to prevent the spread of infected ticks.

Prospects for further research are the search for effective acaricidal drugs, taking into account their toxicity.

Keywords: *Ixodes ticks, spread, risks, transmissible diseases, Ixodidoses of animals*

Introduction

Ixodes ticks (in Latin *Ixodidae*) are carriers of dangerous transmissible infections and invasions, such as Tick-borne encephalitis (TBE), Tick-borne borreliosis or Lyme borreliosis, Tularaemia (in Latin Tularenia), Tick-borne rickettsial pathogens, in particular, query fever (Q-fever) and Marseilles fever (Galat, 2003; Nikiforova, 2007; Luginin, 2011). Tick-borne encephalitis and Lyme borreliosis (Lyme disease), from which both humans and animals suffer, are the most well-known and widespread in Ukraine (Klymniuk, 2017).

The transmission of dangerous pathogens occurs after the *Ixodes* tick bite. However, even a short tick stay on an animal's body can result in its infection. This feature is associated with factors of immunosuppression of the secretion of glands that enter the *Ixodes* ticks during the bite (Afzelius, 1910; Appel et al., 1993). Therefore, we believe, that *Ixodes* are a vector of transmission of dangerous transmissible diseases of humans and animals (Burgdorfer, 1984).

The widespread ticks distribution of the genus *Ixodidae* in the world, including Ukraine, the mosaic nature of their spatial distribution, the adaptive diversity of life cycles, as well as the potential danger as vectors of infectious and invasive diseases of animals and humans make the task of their detailed studying relevant. Thus, it is important to study the dynamics of the number of *Ixodes* in different countries and regions of Ukraine, as well as the structure of the fauna, features of biotope distribution, the development cycle of these ticks, and others. The research on the territory of Ukraine, where the natural center of dangerous transmissible infections and invasions have been registered, is important.

Analysis of recent researches and publications

The studies of Ukrainian scientists V. F. Galat, A. V. Berezovsky, M. P. Prus are devoted to *Ixodidae*. N. M. Soroka (2003) considered and examined invasive animal diseases (Galat at al., 2003); O. V. Nikiforova (2007) analyzed the spread of *Ixodes* ticks as carriers of pathogens of *Borrelia burgdorferi sensu lato*, *Babesia microti*, *Ehrlichia phagocytophila* geno group, *Bartonella spp*., *Rickettsia spot* fever group, *Toxoplasma gondii* in the south-eastern part of Ukraine (Nikiforova, 2007); M. S. Luginin (2011) investigated trophy-consortial relationships of *Ixodes* ticks in forest ecosystems and their role in the spread of pathogens of natural focal diseases in humans and animals (Luginin, 2011); O. E. Galatiuk, O. O. Peredera, I. V. Lavrinenko, I. A. Gernosik (2018) analyzed infectious diseases of dogs (Galatiuk et al., 2018).

Dangerous pathogens of transmissible diseases transmitted by *Ixodes* ticks, is researched and studied in European countries (Steere et al., 1977; Lindgren et al., 2006; Littman et al., 2018; Regional Committee for Europe EUR/

RC68/16 68th session Rome, 2018). So the pathogen *Borrelia burgdorferi* was registered in Germany by Zeman (1997) and Hönig et al. (2011), in Italy – by Rizzoli et al. (2004). Also, Schwarz et al. (2009) made predictions about the spread of *Ixodes* in Germany, and Braga (2012) – in Scotland.

In Ukraine, the pathogen *Toxoplasma gondii* in *Ixodes* ticks of the genus *Ixodes* was detected and studied by M. V. Galat et al. (Galat et al., 2016).

The prevalence of infection and *Ixodes scapularis* Say, its activity season, survival, wintering and development (Acari: Ixodidae) were studied by Zachary C. Polk (Manitoba, Canada, 2017); Meryl P. Littman, Bernhard Gerber, Richard E. Goldstein, Mary A. Labato, Michael R. Lappin, George E. Moore (American College of Veterinary Internal Medicine, ACVIM, 2018).

Researchers analyzed the reasons for the geographical distribution of infected and infested *Ixodes*; developed diagnostic tests to rule out coinfection and other causes of clinical signs that could potentially be associated with Lyme disease; tested in the experiment effective methods for treatment of dogs and other animals for arthritis and nephritis caused by *Ixodes*, as well as developed treatment regimens for *Ixodes* (Littman et al., 2018; Regional Committee for Europe EUR/RC68/16 68th session Rome, 2018).

Purpose. The aim of the study is to analyze the prevalence of *Ixodes* as vectors of dangerous transmissible infections and invasions in Ukraine and to identify risks for their prevention.

Materials and methods of research

For the study, we collected *Ixodes* in the forest park areas of Kyiv and the Kyiv region. We used the conventional method of direct selection of Ixodes in animals. It is based on a selective or complete collection of *Ixodes* mites found on dogs of different breeds. We used a thin wooden board (size 15–20 × 5 cm) with pointed long edges for select *Ixodes* from dogs with short and straight hair. We combed the board against the wool and looked behind the plate for lightened skin under the conditions of *Ixodes*.

In addition, we also searched *Ixodes* ticks conventional method, namely, collecting them from the environment in a simple white cloth material (size 60 x 100 cm). Adults of *Ixodes* (females, males) were collected from vegetation; preimaginal stages (larvae, nymphs) – for combing small dogs. In total, we examined 150 adults, 60 nymphs, and 30 larvae of *Ixodes*, including adult genera *Ixodes* 110 and *Dermacentor* – 40.

The polymerase chain reaction was used to isolate the DNA of *Borrelia burgdorferi* and *Toxoplasma gondii* from *Ixodes* ticks. The results of the reaction were analyzed using special software.

Ixodes ticks were mechanically ground, incubated at 56 °C with the addition of proteinase K for 16 hours. Proteinase K is a non-specific serine protease with high proteolytic activity. The obtained suspension was centrifuged, the supernatant was collected and DNA isolation of pathogens was performed using a special set (according to the instructions for use). This set contained a mixture of required primers that are necessary for DNA replication during amplification.

Results of the research and their discussion

We conducted experimental studies during 2018–2019 in periods of high activity of *Ixodes* ticks – in spring (April, May), and in early summer (June), and

autumn (September, October). According to the results of the research, *Ixodes* ticks of the genera *Ixodes* and *Dermacentor* were found in the forest park zones of Kyiv and Kyiv region. Other genera of *Ixodes* ticks were not registered.

Laboratory tests revealed the presence of DNA of *Borrelia burgdorferi* and *Toxoplasma gondii* in the body of *Ixodes*, larvae and nymphs of the genus *Ixodes*. We did not detect these pathogens in ticks, larvae, and nymphs of the genus *Dermacentor*.

The presence of *Borrelia burgdorferi* in *Ixodes* of the genus *Ixodes* confirms the main natural source of infection with this pathogen in various species of animals and humans in Ukraine and, in particular, in the city of Kyiv and the Kyiv region. At that time, the detection of *Toxoplasma gondii* in *Ixodes* ticks indicates one of the possible sources of infection with the pathogen in animals and humans.

The results of the research indicate that a stable natural center of Lyme borreliosis and toxoplasmosis has formed in the forest-park zones of Kyiv and Kyiv region.

It should be noted that the forest park zones of Kyiv and the Kyiv region have sufficient soil moisture, the almost optimal ambient temperature in recent years, diverse flora and fauna, which contributes to the mass spread of *Ixodes* ticks and creates favorable conditions for the circulation of pathogens Borreliandurg in them.

Investigating the emergence of mites of the genus *Ixodes* and *Dermacentor* from the winter diapause, in recent years there have been earlier periods, and the period of activity lasts until mid-November, which leads to an increase in numbers and, consequently, attacks on animals and humans.

In this regard, the epizootic and epidemic situation of Lyme borreliosis disease and toxoplasmosis in Kiev and the Kiev region in recent years is regarded as unstable. In particular, the level of infection of humans and dogs with the pathogen *Borrelia burgdorferi* remains quite high.

Our results coincide with the data of state laboratories of veterinary medicine, which indicate the detection of *Ixodes* ticks of the genus *Ixodes* pathogens of Lyme borreliosis. In addition, peaks of *Ixodes* ticks attacks on domestic and farm animals causing ixodidosis are recorded.

Currently, the annual dynamics of infection of animals and humans with *Borrelia burgdorferi* and *Toxoplasma gondii* is characterized by a pronounced seasonality associated with the period of activity of ticks of the genus *Ixodes*. It is established that the largest number of attacks of *Ixodes* ticks on dogs and humans is registered in May-June and September-October.

Ixodes ticks bite involving a violation of the integrity of the skin, allergic reactions to foods salivary glands, irritation, and inflammation.

With a significant invasion, ticks cause ixodidosis, which is characterized by the development of anemia and severe stress and is accompanied by loss of appetite and exhaustion, decreased resistance of animals.

It should be noted, that, currently, there are new enzootic areas in the Kyiv region, both at the site of infection of humans and animals (suction of ticks), and the results of studies of *Ixodes* ticks (positive findings). At the same time, natural foci of Lyme borreliosis were found in most forest park areas of the city and region. Therefore, the risk of infecting humans and animals depends on the number of infected and infested

Ixodes ticks in the region and the frequency of contact with them.

First of all, we focused on the features of the biology and ecology of *Ixodes* ticks to determine the risks associated with the spread of *Ixodes* mites and their prevention.

As it is known (Nikiforova, 2007; Luginin, 2011), the development cycle of *Ixodes* ticks covers three active stages: larva, nymph, and imago (female, male). At each stage of their transformation and to maintain their vital activity, *Ixodes* mites find a suitable host to feed on its blood and maintain the temperature they need (Fig. 1).

As a rule, an adult tick needs a large host, a mammal, such as a deer. Larvae and nymph need a small host, such as a rodent, a small bird, and a dog. After feeding, female *Ixodes* ticks hide in vegetation and the upper layers of the soil and lay thousands of eggs, and then die. At the same time, larvae and nymphs go through stages of development to adult directly on animals or also in the environment.

The efficiency of transmission of *Borrelia* in the body of ticks in different individuals ranges from 26.5 to 81.4%. The spirochetes appear in the hemolymph of the mite in the first hours after infectious blood suction on the donor animal, and after 4–6 days in the body of the female mite. They were found not only in the intestine, but also in the brain, Malpighian tubes, and, most importantly, in the salivary glands and eggs.

The nymph bites, in contrast to adults, in the vast majority of cases go unnoticed, which should be taken into account when collecting epidemiology. The same role in the circulation and preservation of the causative agent of Lyme borreliosis belongs to small mammals in which the pathogen experiences an inter-epizootic period. The rodents can be carriers of *Borrelia* for almost their entire lives and play a significant role as a reservoir of infection in nature.

It is noted that some *Ixodes* ticks develop from eggs, two immature stages

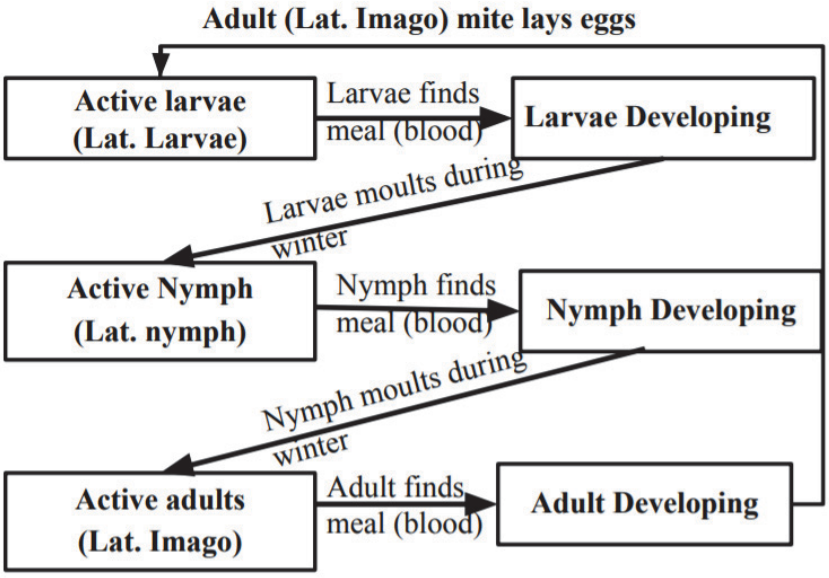

Fig. 1. The life cycle of Ixodes ticks

(larvae and nymphs), to adults, within 1–3 years. Each immature stage in the development of *Ixodes* mites requires feeding on the blood of certain animals, which takes several days. In addition, the environment has a very sensitive effect on their activity and survival. Gilbert et al. (2014) investigated that ticks of the genus *Ixodes*, more than other genera, are sensitive to low temperatures. Therefore, they usually retain their viability by hiding in vegetation, soil (their behavior, known as a quest). In conclusion, scientists found that "questing behavior in response to temperature varies between *I. Ricinus* populations in relation to thermal climate with a higher proportion of the population questing at lower temperatures if they are from cooler climates" (Gilbert et al., 2014).

Randolph et al. (2002) found that these ticks try to avoid drying out, especially at low or high humidity. This conclusion was validated when emergence dates of unfed ticks predicted from a day-degree summation development model coincided precisely with the first appearance in the field of ticks with high fat content (Randolph et al., 2002). It is important to consider these factors to predict the future activity of *Ixodes* ticks or their numbers.

The Geographic Information Systems (GIS) are now increasingly used to make spatially large-scale associations between environmental factors and disease risk parameters. These maps have enabled us to gain insight about diseases ranging, for example, from Encephalitis to Lyme borreliosis, or to focus on the fact that GIS to simulate the dynamics of diseases transmitted by *Ixodes* ticks. The Medical GIS will remain a lasting approach for understanding populations and the world we live in.

The presentation of data in the form of an interactive portal of online maps is an ideal way to convey information to the public. For example, map of "Ticks and tick-borne diseases in South Bohemia and regions of Bavaria 2008–2020" by scientists of University of South Bohemia, VŠB – Technical University Ostrava, Institute of Parasitology, BC AS CR, v.v.i. (http://gisak.vsb.cz/klista-ta/index_en.html). The main purpose of the map portal is to make the data on the occurrence of ticks and tick-borne pathogens in South Bohemia, Lower Bavaria, and Upper Palatinate available to the public (Švec et al., 2009; Honig et al., 2015; Švec et al., 2019; Honig et al., 2019; Zubriková et al., 2020).

To prevent ixodidosis in animals, a variety of chemical drugs (acaricides, insecticides) of domestic and foreign manufacturers and repellents are applied to the skin in the form of aerosols, suspensions, powder, drops, shampoo, as well as collars, tapes. However, some of them are not effective enough, and some are even toxic.

Thus, analyzing the epizootological and epidemiological situation, we should talk about the spread of Lyme borreliosis and toxoplasmosis among humans and animals and the expansion of their vectors, in particular the increase in the number of ticks of the genus *Ixodes* in forest parks of Kyiv and Kyiv region. The main reason for this phenomenon, in our opinion, is climate change and ecology. In addition, there is a set of natural and social factors that create receptive conditions for the reproduction of *Ixodes* ticks. In particular, the mitigation of the climate in Ukraine, the destruction of conifers, and the emergence of small deciduous shrubs with undergrowth and tall grass, as well as severe cluttering of the landscape in some forest parks, the emergence of ro-

dents and intensive construction in suburban areas. Therefore, the importance of preventive measures for Lyme borreliosis and toxoplasmosis in animals and humans, it is timely laboratory diagnosis and specific prevention.

Due to the above, it is necessary to create an operational algorithm for epidemiological surveillance of natural foci of infection transmitted by ticks (Fig. 2).

The algorithm involves the following actions:

- Epidemiological surveillance with *Ixodes* tick-borne borreliosis;
- Monitoring of objects or phenomena;
- Analysis of monitoring results;
- Decision making (development of a plan of preventive measures);
- Implementation of the action plan;
- Monitoring the effectiveness of measures;
- Correction of the monitoring program and the plan of preventive measures;
- If monitoring the effectiveness of measures showed positive results, then we suggest to repeat the epidemiological surveillance with *Ixodes* tick-borne borreliosis, according to the season favorable for the spread of ticks;
- If monitoring the effectiveness of measures showed a negative result, it is proposed to correct the monitoring program and the plan of preventive measures and repeating the algorithm cycle "Implementation of the action plan – Monitoring the effectiveness of measures".

The affiliation of *Ixodes* tick-borne borreliosis to natural focal infections determines the specifics of surveillance, which should be considered as epizooti-

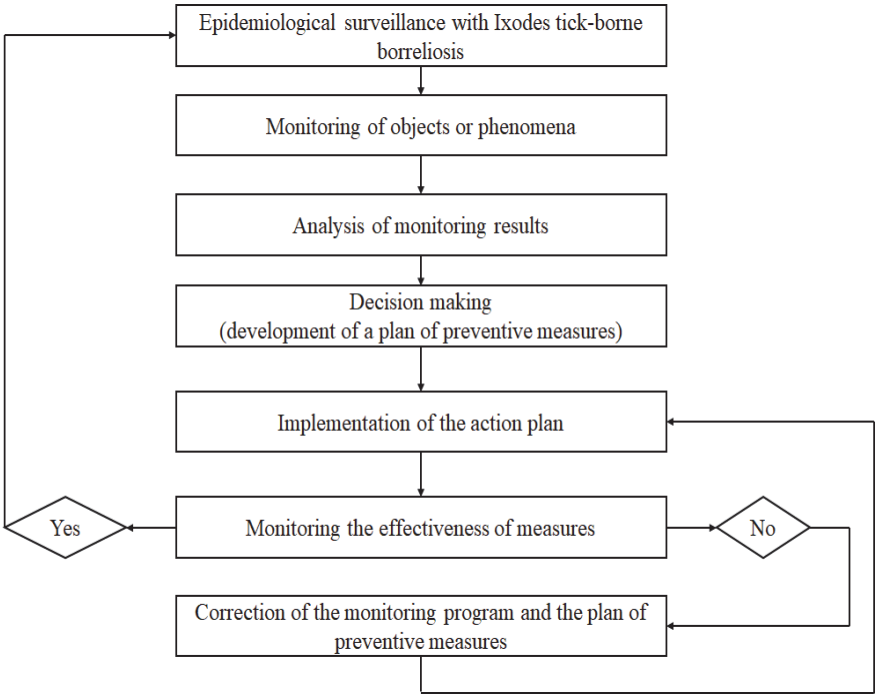

Fig 2. Algorithm for epidemiological surveillance of natural foci of tick-borne infections

ological and epidemiological, including epidemiological, immunological, and zooentomological monitoring.

The epidemiological monitoring involves monitoring the intensity of the epidemic process, dynamics (perennial and seasonal), territorial distribution, and structure of the disease and includes laboratory examination of patients, an epidemiological examination of the case (or suspicion) of *Ixodes* tick-borne borreliosis. The distribution of morbidity around territory is traditionally grouped by administrative principles (cities and districts of the region, districts of the city).

The structure of the disease includes the distribution by sex, age, social and occupational composition with the selection of "risk groups", by clinical forms (1st stage – migratory erythema; 2nd–3rd stages – indicating the leading symptoms and severity of the course).

The epidemiological examination of the case (or suspicion) of *Ixodes* tick-borne borrelios is a compulsory component of epidemiological monitoring. Sanitary and epidemiological station after receiving an emergency notification in case of disease or suspected *Ixodes* tick-borne borrelios (form N 058/o) conducts its epidemiological examination. Collection of information on epidemiological monitoring is carried out according to the data of official accounting documentation – the form N 357/o "Map of epidemiological inspection of the center of an infectious disease" (https://zakon.rada.gov.ua/laws/show/z0115-16#Text).

Conclusions and future perspectives

In the forest park areas of Kyiv and Kyiv region found *Ixodes* ticks and *Dermacentor* genera. Other genera of *Ixodes* ticks have not been registered.

The Laboratory tests revealed the presence of DNA of *Borrelia burgdorferi* and *Toxoplasma gondii* in adults, larvae, and nymphs of the genus *Ixodes*. In adults, larvae and nymphs of the genus *Dermacentor* these pathogens were not detected. The annual dynamics of infection with *Borrelia burgdorferi* and *Toxoplasma gondii* is characterized by a pronounced seasonality associated with the period of activity of ticks of the genus *Ixodes*. The highest number of *Ixodes* ticks attacks on dogs and humans is recorded in May-June and September-October. The presence of *Borrelia burgdorferi* in ticks of the genus *Ixodes* confirms the main natural source of infection with this pathogen in various species of animals and humans in Ukraine and, in particular, in Kyiv and the Kyiv region. At the same time, the detection of *Toxoplasma gondii* in *Ixodes* ticks indicates the presence of one of the possible sources of infection with the pathogen in animals and humans.

The research results show that a stable natural center of Lyme borreliosis and toxoplasmosis has formed in the forest park zones of Kyiv and Kyiv region.

To prevent the spread of infected ticks, in our opinion, it is important:

- timely prevention of animals from ticks;
- research of ticks in the territories of their possible spread;
- analysis of the ecological situation within three or four years in the area where ticks are most common;
- creation of maps with the help of Geographic Information Systems that reflect the dynamics of diseases transmitted by Ixodes mites; public awareness.

Prospects for further research are the search for effective acaricidal drugs, taking into account their toxicity.

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Анотація. *У статті розглядаються вітчизняний та зарубіжний досвід щодо поширення іксодових кліщів – переносників збудників небезпечних трансмісивних інфекцій та інвазій у лісопаркових зонах України і превентивних заходах щодо іксодідозів тварин та людини. Матеріалом для дослідження слугували дані ветеринарної звітності, отримані від державних регіональних лабораторій ветеринарної медицини за 2018–2019 роки. Дослідження проводилися на кафедрі паразитології та тропічної ветеринарії Національного університету біоресурсів і природокористування України. Для досліджень використовували іксодових кліщів, попередньо зібраних у лісопаркових зонах Києва. Аналізувалися результати дослідження іксодових кліщів на наявність збудників небезпечних трансмісивних інфекцій та інвазій, а також лікування собак за іксодідозів.*

Для запобігання поширенню інфікованих кліщів важливими нами визначено своєчасна профілактика тварин від кліщів, дослідження кліщів на територіях їхнього можливого розповсюдження; аналіз екологічної ситуації в межах трьох-чотирьох років на території, де найчастіше розповсюджуються кліщі; створення мап за допомогою Географічних інформаційних систем, що відображають динаміку хвороб, що передаються іксодовими кліщами; інформування населення.

Перспективами подальших наукових досліджень є пошук ефективних акарицидних препаратів із врахуванням їхньої токсичності.

Ключові слова: і*ксодові кліщі, поширення, ризики, трансмісивні хвороби, іксодідози тварин*

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TOXIGENCE OF ANTHRAX VACCINE STRAINS

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Abstract. *The article presents the results of the studying of anthrax strains and Bacillus anthracis-like strains on the formation of toxins. We found that anthrax vaccine strains actively produce exotoxins to the culture fluid. The amount of specific protein is different under the same incubation conditions and depends on the individual characteristics of the microorganism population, because of this, different titers of the toxin are registered.*

Strain B. anthracis K-79 Z (vaccine) with the same number of planting microbial cells and growing on the same culture medium and at the same temperature produces by two orders of magnitude more exotoxin than strains B. anthracis Tsenkovsky II IBM 92Z (virulent), B. anthracis Stern 34F2, (vaccine), B. anthracis 55 (vaccine), B. anthracis SB (vaccine), B. anthracis Tsenkovsky I (vaccine, apathogenic). The amount of exotoxin may change if the pH of the medium changes. The activity of exotoxin production, when the pH changes, depends on the characteristics of the anthrax strain.

Keywords: *anthrax, exotoxin, exotoxin production*

Introduction

Anthrax is a part of the most dangerous infectious diseases common to humans and animals. This disease is common in all mammals, most often among cattle (large and small) and horses. The causative agent is the gram-positive bacterium *Bacillus anthracis*, which forms spores. The infectious dose of the pathogen is only 1–10 spores. They fall victim to the blood of a sick animal or a corpse. The pathogen forms spores that, once in the soil, can persist for more than 100 years. After reaching a favourable environment, the spores become active in 1–6 days, some spores may intensify after 60 days or more (Cizauskas et al.,

2014; Hendricks et al., 2014; Shadomy et al., 2016; Chugh, 2019).

For this reason, the area contaminated with anthrax spores is a factor in the transmission of the disease indefinitely.

During its stay in the soil, the anthrax pathogen periodically restores its population, thus maintaining the infectivity of the territory. To reproduce its population and mass, *B. anthracis* infects animals or humans, and with their corpses, it re-enters the soil and closes the natural cycle of its biology.

Humans can become infected by anthrax via respiratory system (inhalation), gastrointestinal tract, and through the skin (this is the most common form and 95% manifests as a malignant pustule). The inhaled form of anthrax is the most severe, as it often causes septicemia and meningitis with high mortality and the highest risk of man-made spread. The gastrointestinal form of anthrax occurs when consuming meat and meat products of poor quality (Glomski et al., 2007; Hashemi et al., 2015; Owen et al., 2015; Vieira et al., 2017; Mwakapeje et al., 2018).

Between 2,000 and 100,000 cases of anthrax are reported worldwide each year. This disease remains a major health threat in Africa, the Middle East, South America, Central Asia, and Haiti (Doğanay & Eşel, 2008; Abboud et al., 2009; Liu et al., 2020). Anthrax spores can be used as a target for bioterrorism. In 2001, spores of the pathogen were mailed to exceptional individuals in the United States, which caused 22 cases of cutaneous, inhalation, and meningeal anthrax and 5 deaths (Abrol, 2016; Walsh et al., 2018).

Stability of spores for many years, unpretentiousness to the conditions of cultivation leads to human disease or death – all this makes anthrax a primary pathogen of the NIAID category. The pathogen causes 45–90% of mortality even with timely treatment with antimicrobial drugs (Mourez, 2004). High mortality is associated with the rapid development of bacterial infection and the action of tripartite toxin (Crowe et al., 2010).

Both field and vaccine strains of the anthrax pathogen have the ability to produce toxins. *B. anthracis* has two main virulence factors: poly-γ-D-glutamic acid capsule and tripartite toxin (Mock & Fouet, 2001). The capsule plays an antiphagocytic role and allows bacteria to avoid absorption by macrophages (Zwartouw & Smith, 1956). The tripartite toxin contains protective antigen, lethal factor, and oedema factor (Stanley et al., 1960: Stanley et al., 1961). These toxins act on the innate and adaptive immune system and affect the subsequent consequences of the disease (Tournier et al., 2009; Moayeri et al., 2015). Field and vaccine strains clearly differ in the lethality and the amount of toxin production. Field strains produce little toxin during *in vitro* cultivation, but they are more aggressive (predominate lethal and oedematous fractions). Vaccine strains produce more toxin, which is dominated by a protective fraction.

All anthrax vaccines develop antitoxic immunity in a vaccinated animal, but the ability of anthrax microbes to produce an exotoxin is not the same. The stress and duration of immunity (immune protection of vaccinated animals) depend on this property, so studies of the toxigenicity of anthrax strains are relevant.

Analysis of recent sources and publications

Exotoxins of the anthrax pathogen as biotechnological objects are relevant for many scientists in different countries (Friebe et al., 2016).

Studying the protective factor of the antigen and the mechanism of formation have shown that the protective factor may be a new means for controlling cytotoxicity (Kintzer et al., 2009) and studying the complex action of exotoxins as toxic complexes (Lacy et al., 2002). Studying the mechanism of action of a protective exotoxin on a cell against the background of the lethal and oedematous toxin are relevant (Chaudhary et al., 2012; Slater et al., 2013). The use of protective antigen of the anthrax pathogen as an inhibitor of breast cancer cell development has shown the prospects for the development and application of new therapeutic approaches in the fight against tumours (Felix et al., 2020).

Particular attention is paid to the studying of the epizootic situation and disease monitoring worldwide. Creating a unified surveillance system, strengthening cross-sectoral coordination on prevention and control, biological and social strategies for anthrax prevention focuses scientists' attention on endemic regions (Sahoo et al., 2020). Improving anthrax vaccines for humans and animals is an important stage in preventing infection. We are conducting research to find new approaches to the use of antibiotics in the schemes of anthrax vaccines. There is a question of disposable vaccine creation (Clark & Wolfe, 2020).

The development of vaccines, which preserve natural antigenic structures that mimic natural infection, and the studying of the effects of antibodies that neutralize toxins are an important research area (Dumas et al., 2020).

The purpose is to study the properties of toxins formation by anthrax pathogens and *Bacillus anthracis-like* strains.

Material and methods of research

To conduct the experiment we took lyophilized museum cultures of anthrax vaccine strains and *Bacillus anthracis-like* strains: *B. anthracis* Tsenkovsky II IBM 92 Z (virulent), *B. anthracis* K-79 Z, *B. anthracis* Sterne *34F2*, *B. Anthracis* 55, *B. anthracis* SB, *B. anthracis* Tsenkovsky I, *B. anthracoides* 67, *B. Subtilis* 17, *B. cereus* 8035 (virulent), which are stored in collection of microorganisms in the "State Center for Innovative Biotechnology". The methods for studying vaccine strains described by S. G. Kolesov, generally accepted in microbiological practice, were used (1976).

The agglutination reaction and the concentration of the spore suspension for each strain were determined in colony forming units (CFU) by scientific and methodological recommendations "Laboratory diagnosis of anthrax in animals, detection of pathogens from pathological and biological material, raw materials of animal origin and environmental objects" (Skrypnyk et al., 2015). Statistical processing of the obtained research results was performed using the program "Microsoft Excel 2011" for Windows.

The titer of microorganisms toxigenicity in the causative agent of anthrax and *Bacillus anthracis-like* strains was determined using the disc precipitation reaction (DPR) by the method improved by A. I. Zaviryukha (Zaviryukha & Stepanyuk, 1978). Precipitated anthrax serum RP № 3905-14-0403-08, manufactured by Kherson State Biological Factory, was used for the study.

Bacterial cells of vaccine strains and *Bacillus anthracis-like* strains were grown on standard culture media: broth and meat-peptone agar, and injection of 5 and 10 % gelatine at pH 7.2 – 7.6 and a temperature of 37.0 ± 1.0^0 C.

From microbial colonies grown on agar, smears were prepared and stained according to Gram, Mikhin, Olt, Rebiger, Romanowsky-Giemsa, the length and width of microbial cells were measured with a micrometre, and their shape and the presence of spores were determined. The shape and physical characteristics of the colony were studied.

Antigens were prepared by autoclaving from the microbial mass of each strain, which was investigated in the Ascoli reaction and in the DPR with anthrax serum for precipitation. We prepared antigens from the culture fluid of vaccine strains, which were filtered through a bacterial filter and examined by the DPR and Ascoli.

The control was antigens made from bacterial mass and sterile filtrate of culture fluids of anthracoid microorganisms *B. Anthracoides* 67, *B. Subtilis* 17, *B. cereus* 8035 (virulent).

Results of the research and their discussion

Investigated extracellular anthrax toxins, which were obtained by the method mentioned above. The results of the examination by the reaction of Ascoli and the DPR are presented in Table 1.

Our data indicate that the virulent strain *B. anthracis* Tsenkovsky II IBM 92Z and the avirulent *B. anthracis* K-79Z produce extracellular toxin outside the cell membrane in a liquid nutrient medium. The toxin reacts with antibodies of the precipitating anthrax serum in the precipitation reaction. However, the amount of exotoxin in the culture fluid of virulent and avirulent strains is not the same. We noted the ability to produce exotoxin at a titer of 1:32 in vaccine strains of anthrax.

1. The results of studying the sterile filtrates of culture fluids of microbes of the genus *Bacillus*, which were examined by the Ascoli reaction and the disk precipitation reaction

Strain	Ascoli								DPR							
	dilution of culture fluid															
	n	1:2	1:4	1:8	1:1	1:3	1:6	1:128	n	1:2	1:4	1:8	1:16	1:3	1:6	1:128
B. anthracis K-79 Z	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B. anthracis Tsenkovsky II IBM-92 Z	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-
B. anthracis Sterne 34F2	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-
B. anthracis 55	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-
B. anthracis SB	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-
B. anthracis Tsenkovsky I	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-	-
B. cereus 8035	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B. anthracoides 67	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-
B. subtilis 17	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-

Note: n – native fluid; + positive reaction; – negative reaction.

Saprophytic spore-forming bacilli of *B. anthracoides* 67, *B. subtilis* 17, *B. cereus* 8035, and *B. subtilis* 17 do not produce a specific toxin, and therefore their filtrates of culture fluids do not react by precipitation with antibodies of precipitated anthrax serum.

We investigated the impact of pH on the production of exotoxin by anthrax strains, which began with the production of bacterial masses with an identical number of microbial cells – 30.2–30.8 × 10^6 CFU.

Colonies that grown on agar were seeded in a liquid nutrient medium – meat broth with different pH values – 6.5; 8.5; 7.5. They were incubated in a thermostat for 48 hours at 37^0 C. Then we filtered the bacterial mass through bacterial filters type F5. The received filtrates were checked according to DSTU 4483:2005.

We performed the DPR with culture fluid filtrates to determine the anthrax protein titer. The results of the examination are presented in Table 2.

2. Effect of pH on exotoxin production by anthrax vaccine strains, M ± m, n = 10

№	Strain	Number of grown microbial cells, CFU	pH of the medium	Exotoxin titer in the DPR
1.	B. anthracis K-79 Z	30.5 × 106	6.5	8
			7.5	64
			8.5	32
			M ± m	34.66 ± 16.22**
2.	B. anthracis Tsenkovsky II IBM 92 Z	30.7 × 106	6.5	4
			7.5	8
			8.5	4
			M ± m	5.33 ± 1.33**
3.	B. anthracis Sterne 34F2	30.2 × 106	6.5	8
			7.5	32
			8.5	16
			M ± m	18.66 ± 7.05**
4.	B. anthracis 55	30.8 × 106	6.5	8
			7.5	32
			8.5	16
			M ± m	18.66 ± 7.05**
5.	B. anthracis SB	30.5 × 106	6.5	8
			7.5	32
			8.5	16
			M ± m	18.667 ± 7.05**
6.	B. anthracis Tsenkovsky I	30.2 × 106	6.5	8
			7.5	16
			8.5	8
			M ± m	10.66 ± 2.66**

Note: * P < 0.05; ** P < 0.02; *** P < 0.01; **** P < 0.001.

Indicators of Table 2 show that at different pH of the medium, the amount of specific protein in the strains increased differently (1:8–1:64 – *B anthracis* K-79Z, 1:8–1:32 – *B. anthracis* Sterne 34F2, *B. anthracis* 55, and *B.* anthracis SB, 1:8–1:16 – *B. anthracis* Tsenkovsky I*)*. At the same cultivation conditions, a high titer of specific anthrax protein showed the exotoxin *B. anthracis* K-79Z – 1:64 at pH 7.5. Strains *B. anthracis* Sterne 34F2, *B. anthracis* 55, and *B. anthracis* SB at pH 7.5 produced exotoxin at the same level, and it was 1:32. Also, in these strains, the same amount of exotoxin was observed when cultured in a medium at a pH of 6.5, which was 1:8. When the pH was raised to 8.5, the exotoxin purge of these strains was at 1:16. This indicates similar characteristics of these vaccine strains and the effect of pH on the production of exotoxin.

Conclusions and future perspectives of the study

Vaccine strains of anthrax actively produce exotoxins in the culture fluid. The amount of specific protein under the same incubation conditions is different and depends on the individual characteristics of the microorganism populations.

The *B. anthracis* K-79 Z strain with the same number of microbial cells and under cultivation in the same culture medium and temperature conditions produces by two orders of magnitude more exotoxin than *B. anthracis* Tsenkovsky II IBM 92Z, *B. anthracis* Sterne 34F2, *B. anthracis* 55, *B. anthracis* SB and *B. anthracis* Tsenkovsky I.

Vaccine strains of anthrax, which we studied, produce exotoxin when the pH of the medium. Its amount varies depending on the pH of the medium. The activity of exotoxin production when the pH changes depends on the characteristics of the anthrax strain.

The Perspective: we plan to continue the studying of the toxigenic properties of anthrax strains in order to obtain an exotoxin that will be used in the development of new drugs against anthrax in humans and animals.

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Г. А. Завірюха, У. М. Яненко, Н. І. Кос'янчук (2020). ТОКСИГЕННІСТЬ ВАКЦИННИХ ШТАМІВ СИБІРКИ. *Ukrainian Journal of Veterinary Sciences, 11(3): 85–92, https://doi.org/10.31548/ujvs2020.03.009*

Анотація. *У статті представлені результати дослідження штамів сибірки та антракоїдів щодо утворення токсинів. Ми виявили, що вакцинні штами сибірки активно продукують екзотоксини в культуральну рідину. Кількість специфічного білка різна за однакових умов інкубації й залежить від індивідуальних особливостей популяції мікроорганізмів, через що реєструються різні титри токсину.*

Штам B. anthracis K-79Z (вакцина) з однаковою кількістю посіяних мікробних клітин і за вирощування на одному й тому ж поживиному середовищі та за однакової температури виробляє на два порядки більше екзотоксину, ніж штами B. anthracis Tsenkovsky II IBM 92Z (вірулентний), B. anthracis Sterne 34F2, (вакцина), B. anthracis 55 (вакцина), B. anthracis SB (вакцина), B. anthracis Tsenkovsky I (вакцина, патогенна). Кількість екзотоксину може змінюватися внаслідок зміни pH середовища. Активність продукування екзотоксину внаслідок зміни pH середовища залежить від характеристик штаму сибірки.

Ключові слова: *сибірка, екзотоксин, продукція екзотоксину*

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PATHOMORPHOLOGICAL FEATURES OF TESTICULAR TUMORS IN DOGS

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Abstract. *The research was conducted to study the morphological, clinical, and macroscopic characteristics of the different histological types of canine testicular tumors. The material was obtained during a routine surgery for the removal of testicular tumors in dogs in clinics of veterinary medicine in Kyiv. Tumors were divided into sex cord-stromal tumors and germ cell tumors. Among the sex-cord stromal tumors recorded Leydig (interstitial) and Sertoli cell tumors. Macroscopic and histologic characteristics of canine interstitial (Leydig) cell tumor are described. It comprises 12% of all testicular tumors. Interstitial (Leydig) cell tumor has a slower growth compared to other testicular tumors, metastases are not observed. Half of the animals had no clinical signs. Most interstitial (Leydig) cell tumor go together with the pathology of adnexal structures. This is because Leydig cell tumor leads to increased levels of androgens. This fact may cause the pathology of adnexal structures. Neoplasms are recorded both on the right and the left testis. Leydig cell tumor was not related to the cryptorchid testicles. Mostly, tumors are regular oval, brown or gray color, different texture, and size with no signs of necrosis or ulceration. Macroscopic and histologic characteristics of malignant tumors of the testicle are described.*

A macroscopic picture was different and did not have clear meaning with the histological type of tumors. Microscopically a seminoma was characterized by the presence of layers of oval cells with the expressed grainy cytoplasm, surrounded by connective tissue, in which there was significant lymphoid infiltration. Leydig cell tumor is composed of cells that resemble Leydig cells and that are arranged into islands or tubular structures. The polygonal eosinophilic cells with granular or vacuolated, which contains lipids, were detected. Nuclei were a round mesh of chromatin and distinct nucleolus. Sometimes the large cells with 1, 2, or more nucleoli were noted. In Sertoli cell, tumor marked the various spectrum of cages from polygonal to prismatic sustenocytes.

Keywords: *non-germ cell tumors, genital stromal tumors, benign tumors, Leydig cell tumor, sertolioma, seminoma, testicular neoplasms, dogs*

Introduction

Risk factors for cancer in dogs are generally similar in humans, although many questions remain unclear in this regard. The important role of genetic factors is evidenced by the data on the unequal frequency of neoplasms in dogs of different breeds, as well as the fact that tumors of dogs are often observed in purebred animals (about 75%) compared with crossbreed or mixed breed ones (Moulton, 2002; Dobson & Lascelles, 2003). This assumption is not always confirmed, some authors believe that the high incidence of tumors in mongrel dogs is associated with poor living conditions. It has been shown that among breeds of various groups, the highest numbers of sick animals are registered among service (50–70%) and hunting (25–37%) breeds, and the lowest – in Toy breeds (8–28%) (Bernshtein, 2000; Sereda, 2009; Henry & Higginbotham, 2010). It is possible, however, that a greater percentage of service and hunting dogs breeds in the structure of morbidity, particularly tumors, is associated with a more attentive attitude towards them by owners and more frequent visits to veterinary hospitals, given that these dogs are more valuable in terms of economic considerations of their use.

Analysis of recent research and publications

An important endogenous risk factor for tumors is the age of animals: morbidity increases with age and is a well-known fact. According to the age range of diagnosis, tumors of dogs registering throughout their life, even in the first year, however, the largest part of sick animals is observed at the age of 7–12 years. The average age of dogs with testicular cancer is 10 years. Extremely rare neoplasms of the testes are found under the age of 6 years.

One of the factors that increase the risk of testicular tumors is cryptorchidism with an inguinal or abdominal location. Most authors note that the testicles, which have not descended, sertoliomas and seminomas develop more often (Dobson & Lascelles, 2003; Villalobos, 2010). Higher number of tumors register in the right testicles is explained by the fact that they do not descend more often.

The breed predisposition to this pathology is also described. Most often, testicular tumors are recorded in dogs of such breeds as Siberian Laika, Fox Terrier, Afghan Hound, and Sheltie (Bernshtein, 2000).Clinical signs vary and depend on several factors, first of all on the location of the testicles (in the scrotum, groin, or abdominal cavity), the presence of metastasis, and the development of paraneoplastic syndromes. At the same time, testicular neoplasms are found accidentally very often (North & Banks, 2009). The most common clinical manifestation of testicular tumors is feminization syndrome. The development of feminization syndrome depends on the localization of the neoplasm, localization in the scrotum – 16% of cases, in the groin – 50%, and in intra-abdominal location – 70% (Bernshtein, 2000). Moreover, bone marrow hypoplasia with thrombocytopenia, bleeding with anemia and granulocytopenia, gynecomastia and galactorrhea, attractiveness for males, atrophy of the opposite testis, atrophy of the penis with preputial sagging, bilateral symmetrical alopecia in the genital area, inner thighs, abdomen, and chest are also observed (Sereda, 2009).

The prognosis of the disease depends on many factors. The significance of the size of the primary tumor, its location, the presence of regional and distant metastases, the histological type and level of

differentiation of tumor cells, infiltration into the surrounding tissues, and the clinical stage of the disease are considered. However, the opinions about determining the prognostic value of certain characteristics of tumors differ among the researchers. Thus, most authors believe that a tumor size less than 3 cm in diameter in case of absence of metastases is associated with better prognosis (Henry & Higginbotham, 2010; Dobson & Lascelles, 2003), and the presence of metastasis is associated with a poor further prognosis. At the same time, there are opposite opinions: some authors believe that metastases to regional lymph nodes cannot be considered a prognostic factor (Villalobos, 2010).

However, in the literature data, the issues on the frequency of tumors occurrence in dogs, factors of anamnesis, as well as the peculiarities of tumor micro- and macrocharacteristics, which a veterinarian encounters, are not sufficiently highlighted. The studying of the pathomorphological features of testicular tumors is extremely important for an accurate diagnosis.

Purpose. The aim of the work was to study the frequency of diagnosis, clinical morphological, and macroscopic features of various histological types of canine testicular tumors. According to the goal, it was planned to investigate: the pathomorphological features of various histological types of tumors, their clinical manifestation, and the relationships between testicular tumors emerging and anamnestic data.

Materials and methods of research

Experimental materials were obtained during elective operations for testicular tumors removal in dogs at the veterinary clinic "Animal health".

The pathological material was processed according to generally accepted histological methods.

For histological studies, a tumor or part of it was sampled, along with the capsule and surrounding tissues, and placed into a special glass or plastic container with a tightly ground lid. They were fixed in a 10% neutral aqueous solution of formalin according to Lilly's prescription, dehydrated in ethanol of increasing strength, and after chloroform embedded in paraffin. Using a sled microtome, sections of 6–8 μm were obtained. The histological structure of organs and tissues was studied by sections staining with Carazzi's hematoxylin and eosin. The histological slides were examined and pictures were made using an Olympus BX-41 microscope (Goralskij, Radzihovskij, & Zayika, 2018).

Results of the research and their discussion

The priority, when a testicular tumor suspected, is a thorough history taking, which must include data on the dog's behavior, the time of the disease emerging, the rate of tumor development, and visual changes along this process. It is important to find out if the treatment was provided, what kind of treatment, and when it was carried out. During the clinical examination, a general examination of the animal is carried out and considerable attention is paid to the area of the pathological process. The general condition of the animal depends on the type of tumor and its stage, but in most cases, it changed insignificantly. Decreased appetite and apathy are observed in 18% of dogs. Testicular tumors are accompanied by pain in 16%.

Palpation was used to determine the location of the testicles. Their size, densi-

ty, shape, consistency, and mobility were determined. Examining regional lymph nodes, attention was paid to their size, density, mobility, and pain. Additionally, a rectal examination was performed to determine the condition of the prostate.

Morphological studies are the main diagnostic procedure to establish the nature of the tumor and its histological type. These data in combination with other methods allow determining the stage of the disease and choosing the most effective method of treatment. We used two types of morphological studies: cytological and histological.

Testicular neoplasms are easily amenable to surgical removal followed by histological examination. Tumors up to 1 cm in diameter were examined completely. For a homogeneous tumor, 3 samples were taken along a line passing through the center of the tumor. In the case of macroscopic inhomogeneity, samples were taken from all areas, as well as along the median line. In all cases, it is important to take a sample at the border of the healthy and affected tissue. Together with the tumor tissues, a histological examination of regional lymph nodes was carried out, which provided a full characterization of the level of the pathological process development. Dogs with poorly differentiated tumors had a poor prognosis compared to animals with tumors made up of highly differentiated cells.

According to the principles underlying the classification of testicular tumors of dogs (Meuten, 2002), tumors that occurred in the studied random sample were divided into sex cord-stromal tumors and germ cell tumors. Three types of testicular tumors were most often found in small domestic animals: a tumor from Leydig cells – leydigioma, a tumor from Sertoli cells – sertolioma, and a tumor of the spermatogenic epithelium of the seminiferous tubules – seminoma.

An important prognostic factor is the histological structure of the tumor. Thus, an animal diagnosed with sertolioma or leydigioma has a more favorable prognosis than animals diagnosed with seminoma. Some authors also consider unfavorable factors such as the degree of vascular and tissue invasion, lymphoid infiltration, the size of the zones of necrosis.

Leydigioma is an interstitial cell tumor that develops from Leydig cells. Leydig cells are hormone-producing cells located between the seminiferous tubules in the testes. They produce the hormone testosterone. Also, in small amounts, they synthesize female sex hormones estrogens and progestins. Leydig cells are stimulated by luteinizing hormone, which is produced by the hypothalamic-pituitary system. Leydigioma can secrete testosterone, an excess of which leads to prostatic hyperplasia and the formation of adenomas of the hepatoid glands.

The tumor belongs to non-germinogenic ones that can cause feminization syndrome. In males observing libido reduction, mastopathy development, prepuce tissue swelling, testes size decreasing, becoming flabby, but spermatogenesis is preserved.

Changes in the skin and the hairline are usually started as the shedding period increasing. The coat becomes dull and brittle, alopecia develops, which are mainly localized on the sides. In most cases, the tumor is benign, very rarely metastasizes.

Leydigiomas most often are not accompanied by an increase in testes volume, but they can be detected with scrotum ultrasound examination. Leydigiomas made up 12% of all testicular neoplasms and ranked third after seminomas and sertoliomas. Leydigiomas

occur, as a rule, in the prolapsed testes, and in 15% of cases both testes can be affected. If only one testis is affected, then the other one atrophies.

The neoplasms were mainly represented by one node, but there were also cases when several nodes form were registered in one testicle.

Tumor consistency is soft, gray in color with no signs of ulcers or necrosis.

Microscopically tumor consists of atypical cells that are very similar to their "normal cell analogues". Tumor cells are large, round, or polygonal, sometimes hexagonal, have eosinophilic granular cytoplasm, as well as a small or medium nucleus located in the center. Chromatin reticular, nucleoli are well-defined. Cell boundaries are often not visible. Sometimes large cells with one, two, or several nucleoli were noted. In some cases, spindle-shaped cells with small nuclei and eosinophilic granular or vacuolated cytoplasm were observed. Pronounces cell pleomorphism and mitotic activity was noted.

Cytological diagnosis of testicular tumors is well developed and has a high sensitivity for the diagnosis of each individual type (Fig. 1).

Sertolioma is a tumor of Sertoli scaffold cells (sustenocytes), which perform a trophic function and produce proteins that bind androgens. Most sertoliomas are benign, although morphologically benign and malignant forms do not differ. The malignancy of the process is considered by the presence of metastases. Sertoli cells have testosterone and estradiol receptors and are involved in the regulation of spermatogenesis.

Macroscopically, the tumor is presented in the form of a node with clear contours (Fig. 2). Very often the tumor is surrounded by a connective tissue pseudocapsule.

The color of the tumor is predominantly whitish or whitish-yellow.

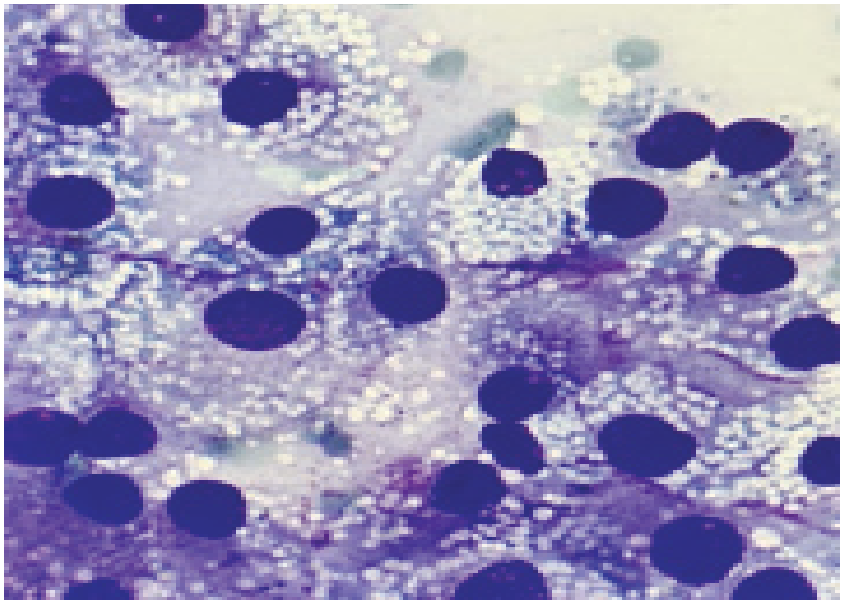

Fig. 1. Cytological structure of leydigioma. Tumor cells with large nuclei and vacuolated cytoplasm

Microscopically the proliferation of tumor cells in the interstitial tissue is detected. Sertolioma cells form sheets and cords. The tumor is mitotically active. Dystrophic changes were observed in the epithelium of the seminiferous tubules. The dysfunction of spermatogenesis is considered by a change in the level of maturity and shape of sperm. The tumor is accompanied by secondary inflammation very often. Tumor tissue vascularization is predominantly moderate.

The tumor has a tubular structure (small tubes separated by thin fibrous connective tissue layers). Glandular structures are visualized in it, which have two types of cell structure (cells with optically empty cytoplasm and cells with hyperchromic nuclei and mitosis presence).

The histological structure of sertolioma was different. A wide range of cells from epithelioid to spindle-shaped resembling fibroma prevailed. Most of the tumors consisted of sustenocytes of various shapes: from polygonal to high prismatic, which formed tubules or solid areas. The tumor cells were eosinophilic, with vacuolated cytoplasm, a rounded or oval nucleus with a delicate chromatin mesh, and a small nucleolus (Fig. 3).

Tumor cells have a clearly distinguishing nuclear structure and a pronounced rim of the cytoplasm. The nuclei are large, characterized by moderate polymorphism, nucleoli are visible in them. In the structure of the tumor tissue, the cells are located chaotically, sometime they lie freely in tubules lumen as small groups. Signs of cellular atypism were observed in the form of nuclear and cellular polymorphism.

The tumor stroma is represented by fibrous connective tissue layers, in which the slit-like, often collapsed vessels are located.

Seminoma is a highly malignant tumor, most often found in cryptorchids. In advanced cases, the tumor occupies a large volume of the abdominal cavity, is adherent to surrounding tissues, and metastasizes to regional lymph nodes. It

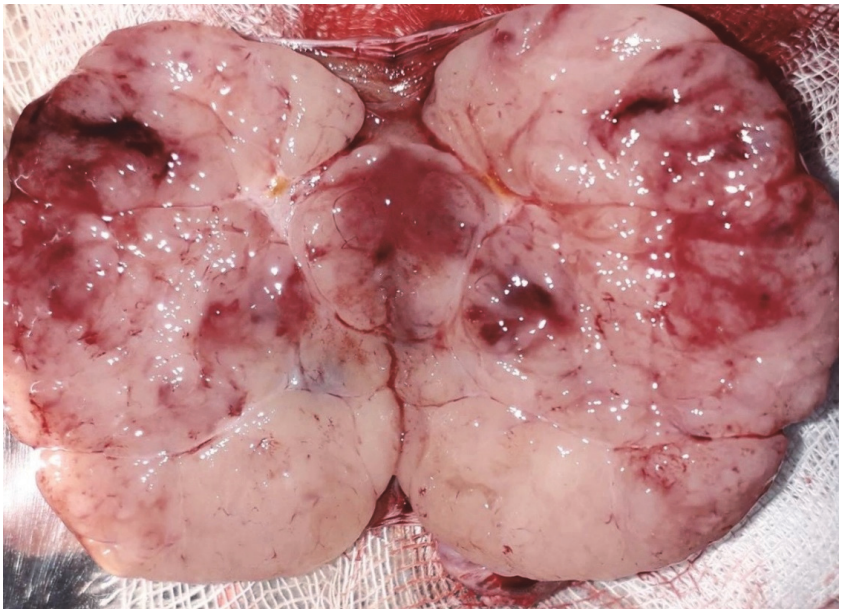

Fig. 2. Sertolioma. Macropreparation

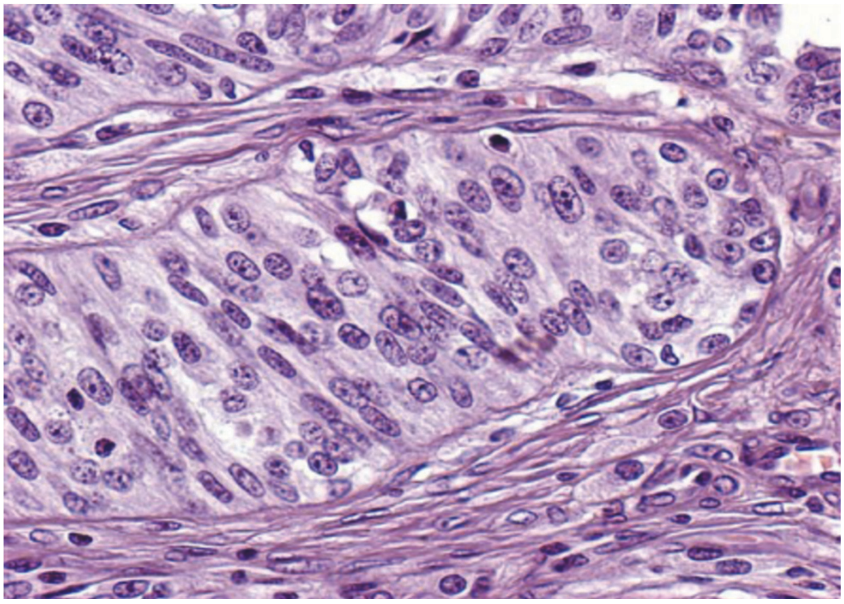

Fig. 3. Histological structure of sertolioma: hyperplasia of spindle cells with hyperchromic nuclei. 400 x.

is possible to observe the bilateral affection or affection of one of the testicles. To suspect seminoma is possible by the appearance of the affected organ. A palpating node or nodes are located in the testicular tissue under the outer shell or inside the organ. The tumor is mostly painful, accompanied by secondary inflammation, and characterized by limited growth in the form of a node or diffuse growth with complete organ affection.

The initial localization of the neoplasm is the seminiferous tubules, but in the case of testicular stromal invasion, it acquires a diffuse form.

Macroscopically, the testicle is enlarged, the tumor has no clear contours, the surface is bumpy, thin-walled cysts were palpated under the capsule. The consistency depends on the presence and number of necrosis foci. Section showed that the neoplasms were loose, swollen, looked like multiple cavities filled with viscous grayish-white pus on the periphery, and in the central part – with dark red bloody fluid. The central part had significant areas of hemorrhage and necrosis. Tissue vascularization was poor. Stroma swelling (from moderate to pronounced) with areas of hemorrhage was observed. Spermatogenesis in the tubules was significantly weakened or completely absent in partly preserved tissue near the site of tumor localization.

In the tumor growth zone atypical, polymorphic cells of epithelioid or polygonal form with rounded often large nuclei with various figures of mitosis, including atypical ones, are detected. In some fields of vision multiple nucleoli in the cells are visible (Fig.4).

In the other fields of vision, sometimes in the capsule, sometimes in places outside of it, there are fields and groups of monomorphic cells with light cytoplasm and clear boundaries of the cell wall. The cytoplasm is vacuolated or optically transparent. Vacuolization of the cytoplasm indicates the presence of

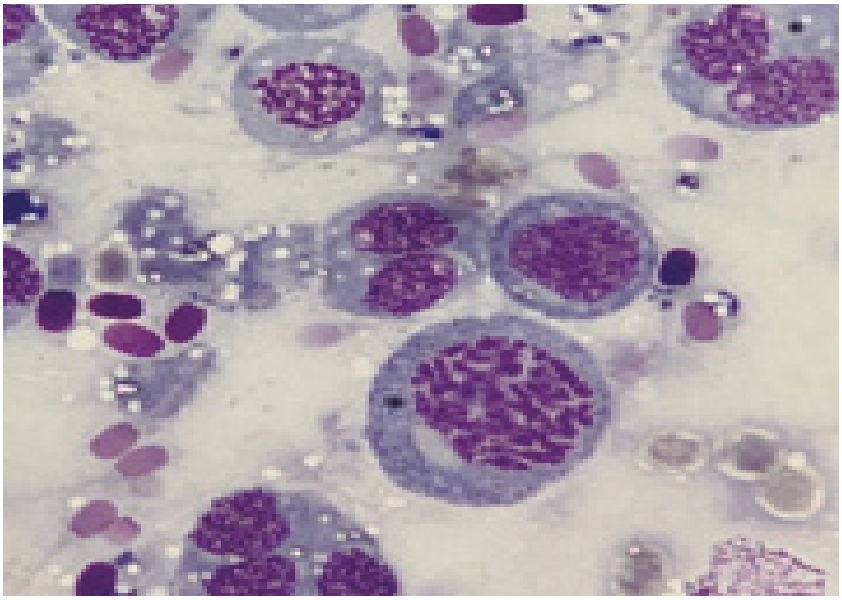

Fig. 4. The cytological structure of the seminoma

degenerative changes in the cells. The cells are located diffusely or in the form of small complexes bounded by fibrous tissue, in which a large number of mature lymphocytes and small foci of necrosis are determined.

In the process of progressive tumor tissue proliferation, violations of the stromal-parenchymal ratio observed. Lymphocytic accumulations were shown in the tumor stroma.

As a result of the seminoma development, the adjacent areas of the organ undergo changes as well. As a result of pressure from the tumor on healthy tissue, circulatory disorders, edema develop followed by disruption of the structure of the spermatogenic epithelium and loss of its functional activity take place. In some cases, the tumor tissue invasion into the capsule was also noted. In this case, the invasion of tumor elements into the skin of the scrotum is observed, the tumor becomes immobile, areas of inflammation and ulcers are traced in the skin.

Conclusions

1. The ratio between benign and malignant neoplasms of the canine testicles was 1:4.2.
2. The prognosis of the disease and the appointment of postoperative chemotherapy depend on the histological verification of the tumor.
3. The largest proportion among all tumors of canine testicles in the studied samples were germ cell tumors, namely seminomas, which were characterized by malignant course prevalence. Among benign neoplasms, Leydigiomas and sertoliomas were observed.
4. The right testis is more often affected. Half of the cases were recorded in the testicle that did not descend with an abdominal location.
5. No dependence on the consistency or shape of tumors on their histological type was found.
6. Carrying out the cytological studies, it is important to remember that in 40%

of dogs, multiple tumors develop in the testicles both of the same type and different (seminoma and sertolioma, seminoma and Leydigioma, etc.). In this regard, a biopsy of all testicular tumors found in the animal by palpation or ultrasound is required.

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Анотація. *Проведені дослідження з вивчення частоти діагностування, морфологічних, клінічних та макроскопічних особливостей різних гістологічних типів новоутворень сім`яників собак. Матеріал для досліджень був отриманий під час планових операцій із приводу видалення новоутворень сім'яників у собак у різних клініках ветеринарної медицини міста Києва. Пухлини були розподілені на пухлини строми статевого тяжу та герміногенні пухлини. Серед пухлин строми статевого тяжу реєстрували лейдигому та сертоліому. Описано макроскопічну та гістологічну характеристики лейдигоми у собак, яка становить 12 % від усіх новоутворень сім'яників. Лейдигома має дещо повільніший ріст у порівнянні з іншими пухлинами сім'яників, метастазів не відмічають. Більшість лейдигом супроводжуються патологією парааанальних залоз. Новоутворення реєструють, як на правому, так і на лівому сім'яниках. Переважно пухлини правильної овальної форми, бурого або сірого кольору, різної консистенції та розміру без ознак виразок чи некрозу.*

Описано макроскопічну та гістологічну характеристики злоякісних пухлин сім›яника собаки. Макроскопічна картина була різною та не мала чіткого зв›язку з гістологічним типом новоутворень. Мікроскопічно семінома характеризувалась наявністю пластів мономорфних овальних клітин із вираженою зернистою цитоплазмою, що були оточені сполучною тканиною, серед якої знаходили значну лімфоїдну інфільтрацію. Мікроскопічно лейдигома складається із гландулоцитів різного ступеня зрілості, які розташовані у вигляді тяжів, пучків, пластів. Спостерігають полігональні клітини з еозинофільною зернистою або вакуолізованою цитоплазмою, що містить ліпіди. У сертоліомі відмічали різноманітний спектр клітин від полігональних до призматичних сустеноцитів.

Ключові слова: *недрібноклітинні пухлини, генітальні стромальні пухлини, доброякісні пухлини, лейдиго-клітинна пухлина, сертоліома, семінома, новоутворення яєчок, собаки*

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PECULIARITIES OF COLLECTION AND CRYOPRESERVATION OF STALLION SPERM

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Abstract. *Horse breeding in Ukraine has always been an industry of national importance, the functional orientation of which has changed because of the development of socio-economic relations. Currently, the role and importance of horses in state farms is complex nature. Breeding horses are used to improve existing and create new, more advanced breeds that would meet the requirements of European and world standards.*

Improving the efficiency of reproduction technologies in horse breeding and further progress of breeds is impossible without the implementation of methods of artificial insemination of horses, including cryopreservation of sperm.

Multiple improvements have been achieved in the past 10 years with regard to utilization and processing of frozen stallion semen, use of alternative cryoprotectors and new commercially available extenders that allow better cell protection during freezing.

It is analyzed that there is only one licensed cryogenic sperm bank of certified sports stallions in Ukraine, which is located in Zhashkiv Equestrian School, from where sperm is transported abroad. It contributes to the improvement of the existing gene pool of horses in our country and promotes the industry. The technological process of selection and cryopreservation of sperm in breeding stallions of the Belgian sport breed in the Zhashkiv Equestrian School of Cherkasy region is investigated. The latest methods for selection and cryopreservation of stallion sperm are presented. It has been investigated that cryopreservation using pre-cooling of sperm for 2.5 h and subsequent rapid freezing in liquid nitrogen vapors is an effective method for maintaining the fertilizing ability of stallions. It is established that following the above rules of selection, evaluation, calculation, dilution, packaging, cryopreservation, and storage of sperm of high-value stallions, the number of sperm banks might be increased. This popularizes the industry, allows to increase the number of farms for breeding horses, and to develop scientific developments in biotechnology and reproduction of these animals.

Keywords: *stallions, phantom, mare, artificial vagina, semen, diluent, packaging, labeling, cryopreservation*

Introduction

Horse breeding in Ukraine has always been an industry of national importance, the functional orientation of which has changed because of the development of socio-economic relations. In the conditions of reforming the agro-industrial complex and transition to a market economy, the development of horse breeding will contribute to the improvement of economic spheres. Currently, the role and importance of horses in state farms has a complex nature. Horse breeding is used to improve existing and create new, more advanced breeds that would meet the requirements of European and world standards (Atroschenko, 2008; Vasileva, 2007). First successful cryopreservation was carried out in the early 20th century using bull semen, and the first effective insemination using frozen stallion semen was performed in the United States of America in 1957 when the semen was taken from the epididymis of the stallion (Gopka, Homenko, & Pavlenko, 2004).

Analysis of recent researches and publications

In the modern practice of horse breeding, obtaining sperm by an artificial vagina for cryopreservation, or insemination with fresh (native) or freshly diluted sperm is a common practice. In recent years, various countries have accumulated considerable experience in working with freshly diluted cooled sperm, developed methods of cryopreservation, which contribute to maintaining high fertility of sperm. Several technologies for cryopreservation of stallion sperm have been developed and are constantly used. Each of them includes many stages that affect the quantitative and qualitative indicators of sperm (Nevzorova, 2011).

The use of frozen sperm allows to determine the timing of insemination, because sperm can be sent at any time before ovulation, and therefore available for insemination at the optimal time (Gopka, Homenko, & Pavlenko, 2004). It also allows avoiding bacterial contamination of sperm during natural mating, which leads to embryonic mortality, decreasing impregnation capacity during subsequent sexual cycles, and the birth of weak or non-viable offspring (Deyaki putannya rozvutky kinnoi galyzi, 2010). Existing ways of sperm freezing differ significantly in technological methods, in methods of preparation for cryopreservation, solvents and media, freezing techniques, method of storage, defrosting, and using (Nevzorova, 2011).

Nowadays interest in horse breeding is growing. This is evidenced by scientific publications of many scientists, laws, and orders on the reorganization of the industry, the creation of new horse farms (Pro stvorennya derzhavnoi ystanovu "Derzhavna durekcia z ypravlinnya, kontrolyu ta rozvutky konyarstva Ukrainu, 2010; https://ec.europa.eu/food/animals/semen/equine_en). At the same time, due to the development of the private sector of the economy, the need for horses not only for work but also for sports, in order to meet the aesthetic and cultural needs of the population has increased.

Currently, there is only one certified horse semen collection center in Ukraine (https://www.pilchuckvet.com/articles/freezing-stallion-semen). This number is due to insufficient logistical and scientific support of the industry, which inhibits the realization of the productive potential of this species of animals and still remains unresolved. Therefore, this issue is relevant to both practitioners and scientists.

The purpose of the study is to develop an algorithm for work with sperm of a stal-

lion of Belgian sport breed, to get acquainted with the newest methods for selection and cryopreservation of stallion sperm.

Materials and methods of research

The research was conducted at the Zhashkiv Equestrian School, during 2020 with 5 clinically healthy stallions of the Belgian sport breed at age from 15 to 19 years.

Collection of material (sperm) was conducted on an artificial vagina "Hannover" using a phantom of the same company twice a week, in compliance with all sanitary requirements, in the morning before feeding the animals. Sperm were macroscopically evaluated for volume, color, odor, and impurities. Microscopic evaluation included determination of the number of living, dead and pathological forms of sperm cells (using automatic software from Minitube), concentration (using the device SpermaCue from the same company). Testing the activity of sperm was performed in the "crushed drop" preparation in compliance with all requirements. INV 96 and INRA Freeze from IMV Technologies were used to thin the sperm, and Maxifreeze medium was used to protect the sperm from the stress of centrifugation. For marking straws an automatic printer EasyCoder for straws of 0.5 and 0.25 ml from Minitube was used. Sperm packing into straws was performed using an automatic packing machine MPP Uno, and the process of freezing semen using a Computer controlled rate freezer 14S and software from Minitube.

Results of the research and their discussion

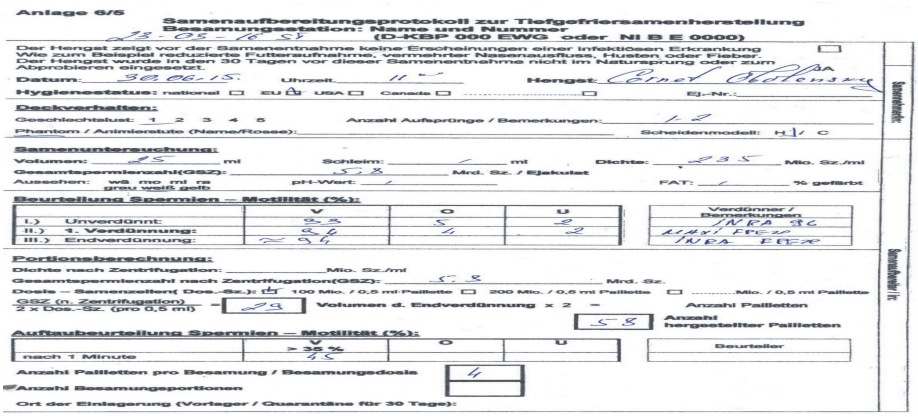
Today there is only one licensed cryogenic sperm bank of certified sports stallions in Ukraine, which is located in Zhashkiv Equestrian School. From that School, sperm is transported abroad (https://www.pilchuckvet.com/articles/freezing-stallion-semen). It contributes to the improvement of the existing gene pool of horses in our country and promotes the industry.

One portion of ejaculate from an artificial vagina can inseminate several mares. This minimizes the spread of diseases and allows to store sperm in liquid nitrogen at a temperature of 196 ℃ for a long time, and the sperm bank will allow you to store sperm of highly breeding offspring and at any time to have access to the best stallions or to sell semen abroad.

Algorithm for working with stallion semen:

1. Collection of ejaculate.
2. Evaluation of ejaculate quality:
   – macroscopic;
   – microscopic.
3. Determining the number of sperm.
   – requirements for native;
   – requirements for frozen.
4. Dilution:
   – sperm dilution medium;
   – description of the process.
5. Packing semen into straws (volume 0.5 ml).
6. Freezing (cryopreservation) of sperm:
   – requirements;
   – description of the process.
7. Storage.

The protocol specifies the main collection parameters (date, time, nickname, number of mounts, number of jumps, use of a phantom or false animal), ejaculate volume, color, odor, mucus, concentration, sperm activity, pathological forms, used solvents for sperm. The protocol also, indicates the number of doses that can be made from this ejaculate, the number of ready doses and straws with semen, and storage location.


Anlage 9/5

Samenaufbereitungsprotokoll zur Tiefgefriersamenherstellung

Besamungsstation: Name und Nummer

(D-KBP 000 EWG oder NI B E 0000)

Der Hengst zeigt vor der Samenentnahme keine Erscheinungen einer infektiösen Erkrankung

Wie zum Beispiel reduzierte Futteraufnahme, vermehrter Nasenausfluss, Husten oder Fieber.

Der Hengst wurde in den 30 Tagen vor dieser Samenentnahme nicht im Natursprung oder zum Abprobieren eingesetzt.

Datum: 30.06.15. Uhrzeit: 11 Hengst: Cornet Obolensky

Hygienestatus: national EU USA Canada Ej.-Nr.:

Deckverhalten:

Geschlechtslust: 1 2 3 4 5 Anzahl Aufsprünge / Bemerkungen: 1-2

Phantom / Animiersstute (Name/Rasse): Scheidenmodell: H1/ C

Samenuntersuchung:

Volumen: 25 ml Schleim: ml Dichte: 2.3.5 Mio. Sz./ml

Gesamtspermienzahl(GSZ): 5.8 Mrd. Sz. / Ejakulat

Aussehen: weiß milchig rahmig grau wässrig gelb pH-Wert: FAT: % gefärbt

Beurteilung Spermien – Motilität (%):

	V	O	U	Verdünner / Bemerkungen
I.) Unverdünnt:	93	5	2	IMRA 96
II.) 1. Verdünnung:	96	4	2	IMRA FFCM
III.) Endverdünnung:	94			IMRA FFCM

Portionsberechnung:

Dichte nach Zentrifugation: Mio. Sz./ml

Gesamtspermienzahl nach Zentrifugation(GSZ): 5.8 Mrd. Sz.

Dosis – Samenzellen(Dos.-Sz.): 100 Mio. / 0,5 ml Paillette 200 Mio. / 0,5 ml Paillette Mio. / 0,5 ml Paillette

GSZ (n. Zentrifugation) / 2 x Dos.-Sz. (pro 0,5 ml) = 29 Volumen d. Endverdünnung x 2 = Anzahl Paillletten

5.8 Anzahl hergestellter Paillletten

Auftaubeurteilung Spermien – Motilität (%):

	V	O	U	Beurteiler
nach 1 Minute	> 35 % 45			

Anzahl Paillletten pro Besamung / Besamungsdosis 4

Anzahl Besamungsportionen

Ort der Einlagerung (Vorleger / Quarantäne für 30 Tage):

Samenhalter

Samenhalter rc

Fig. 1. Cartogram of the humus content of the field with the content of physical clay 45–50%

The School provides each buyer with a freeze report, which shows the data on the stallion's semen (Fig. 2).

According to the protocol, a study was conducted on the collection, evaluation, and cryopreservation of stallion sperm. The first stage was the collection of semen from stallions.

Stage 1. Collection is carried out twice a week, in compliance with all sanitary requirements, in the morning before feeding the animals in the arena, which is well lit, the walls are made of a material that is easy to clean and disinfect, the room is equipped with a phantom for a stallion, model Hannover,

LLC «Zhashkiv Equestrian Sports School»

EQUINE SEMEN COLLECTION CENTRE

23-03-16 ST

FREEZING REPORT

STALLION: CORNET OBOLENSKY

RASSE: Belgian (BWP)

Age at the time of testing: 19 years

LIFE №: 056-002-W001 84083

FREEZING PERIOD: 01.03. 2018 - 10.102018

Parameters fresh semen	
Average number of vaginal leaps:	1-2
Average volume of filtered semen (in ml):	30
Average concentration (in million of spz/ml):	200
Total number of spermatozoa per ejaculate (in milliards):	6,0
Total morphologic normal (%):	91%

Parameters freezing semen	
Number of frozen ejaculates:	42
Average number of straws produced by ejaculate canned:	32-40
Average number of doses produced by ejaculate canned:	4-7
Quality of semen when defrosted (% of motile spermatozoa):	74
AVERAGE CONGELATION(M/ml) / VELOCITY Mic/sec / % PROGRESSIVE MOT:	365 / 50 / 43
Number of ejaculation canned:	42
Color of straws:	grey

10.10.2018 Dr. O.Migovich

EQUINE SEMEN COLLECTION CENTRE

19200 Zhashkiv, UKRAINE

Fig. 2. Sperm Freeze Report

Fig. 3. Phantom for collecting sperm from a stallion

which is covered with a special layer before each mount to prevent contamination by microorganisms (Fig. 3).

The floor around the phantom is covered with special rubber mats in order to prevent injuries. To stimulate a clear manifestation of the male's sexual reflexes, during sperm collection, there is a mare in heat behind the barrier (Fig. 4).

The arena is equipped with a special window to the laboratory for the transfer of ejaculate for further evaluation. The artificial vagina Hannover from Mini-

Fig. 4. Sperm collection

tube is used for collecting sperm which consists of a small valve, an inner cover, rubber rings, and a leather handle.

Before collection, the artificial vagina is pre-disinfected, heated with water to a temperature of 55 °C, and at the time of taking it should be 40–42 °C. A sterile, vaseline-lubricated plastic bag is placed on the prepared artificial vagina, which is tied at the exit end (serves as a sperm collector). The bandaged end is cut after collection, and the semen is transferred to a special filtering vial that separates the semen from the secrets of the vesicles (Fig. 5).

Stage 2. The laboratory assistant conducts the evaluation of the obtained ejaculate, which includes organoleptic evaluation: volume, color, odor, the presence of impurities; microscopic: motility, density, concentration, pathological forms of spermatozoa. Macroscopic evaluation of ejaculate is performed after semen filtration from the secrets of the vesicles in a graduated vessel for filtering the semen of the stallion.

Microscopic evaluation of sperm. Sperm motility is determined in the "crushed drop" preparation simultaneously with the determination of sperm density. Then the concentration of semen is determined using a device SpermaCue (Minitube). Determination of live, dead, and pathological forms of spermatozoa is carried out using automatic software from Minitube.

Stage 3. Calculation of the number of sperm doses according to the formula:

$$\frac{\text{GSZ } (N.Z.)}{2\text{xDos.}-\text{Sz.(pro 0.5 ml)}} = 29 \text{ V. d. E.} \times 2 = 58$$

V. d. E. ×2 = 58 A. S.,

where: GSZ (N. Z.) – concentration of semen in the ejaculate for centrifugation;

Dos.–Sz. (pro 0.5 ml) – sperm dose (100 million in straws 0.5 ml);

V. d. E. – volume of the final dilution;

Fig. 5. Obtained ejaculate

A. S. – number of straws (in our case 58) (Fig. 1).

Requirements for sperm of stallions for artificial insemination (European standard):

fresh semen: at least 300 million spermatozoa with rectilinear translational motion;

cooled semen (diluted / fresh):

- at least 300 million spermatozoa with a rectilinear translational motion at the time of breeding.

- spermatozoa with rectilinear translational motion at the time of insemination not less than 35%.

Diluted semen and semen intended for transportation:

- dose of sperm: at least 600 million spermatozoa with rectilinear translational motion during dilution;

- spermatozoa with rectilinear translational motion at the time of insemination not less than 35%.

Frozen semen:

- dose of sperm: at least 35% of spermatozoa with rectilinear translational motion after thawing;

- at least 250 million spermatozoa with rectilinear translational motion per dose for artificial insemination after thawing.

Stage 4. Dilution of stallion semen:

solvent for sperm INRA 96 in a dose of 1:1 and INRA Freeze is added to the freshly obtained material (Fig. 6); mixing thoroughly with smooth movements;

Fig. 6. Solvent for stallion semen

adding 4 ml of Maxifreeze medium, which protects sperm from centrifugal stress;

centrifugation of ejaculate at 2800 rpm for 20 minutes.

After the sperm deposition procedure, the maximum amount of supernatant (solvent and sperm plasma) is taken.

Step 5. Packing semen into straws (capacity 0.5 ml) using a packaging machine from Minitube MPP Uno. The date of semen collection and the stallion's number are indicated on the sequin. Straws are labeled with an Easy-Coder automatic printer.

Step 6. Cryopreservation: semen for pre-cooling is placed in a refrigerator at a temperature of +4 ℃ on a special plate for 2.5 hours. After that, the straws are moved to the Computer controlled rate freezer 14S, which has specially adapted software from Minitube for quick freezing of stallions. The duration of freezing in this device is 7 minutes to a temperature of -120 ℃. One dose of semen must be thawed and evaluated according to the protocol parameters.

Step 7. After rapid freezing, the semen for long-term storage is transferred to a cryogenic storage Dewar (temperature – 196 ℃) and stored for 60 days in quarantine. Then, the semen is transferred to a storage room in cryogenic storage Dewar.

Thus, following the above rules of selection, evaluation, calculation, dilution, packaging, cryopreservation, and storage of sperm of high-value stallions can increase the number of sperm banks. This promotes the industry, allows to increase the number of farms for horse breeding, and to develop scientific developments in biotechnology and reproduction of these animals.

Conclusions and future perspectives

The algorithm for manipulation with the semen of stallions according to the European protocol is developed.

Cryopreservation using pre-cooling of sperm for 2.5 h and subsequent rapid freezing in liquid nitrogen vapor is an effective method to preserve the fertilizing ability of stallion semen.

Increasing the number of sperm banks of high-value stallions promotes this industry and will increase the number of farms for these animals breeding.

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Анотація. *Конярство в Україні завжди було галуззю загальнодержавного значення, функціональна спрямованість якої змінювалась в залежності від розвитку соціально-економічних відносин. Нині роль і значення коней у господарствах держави має комплексний характер. Племінних коней використовують для поліпшення існуючих та створення нових, більш досконалих порід, які б відповідали вимогам європейських і світових стандартів.*

Підвищення ефективності технологій відтворення у конярстві і подальший прогрес порід неможливі без втілення методів штучного осіменіння коней, в тому числі кріоконсервації сперми.

За останні 10 років було досягнуто багатократних покращень щодо зберігання та переробки замороженої сперми жеребців, використання альтернативних кріопротекторів та нових комерційних розширювачів, які забезпечують кращий захист клітин під час заморожування.

Проаналізовано, що в Україні є лише один ліцензований кріогенний банк сперми сертифікованих спортивних жеребців, який знаходиться в Жашківській кінно-спортивній школі, звідти транспортують сперму за кордон. Він сприяє покращенню існуючого генофонду коней у нашій державі та сприяє популяризації даної галузі. Досліджено технологічний процес відбору та кріоконсервації сперми племінних жеребців Бельгійської спортивної породи в Жашківській кінно-спортивній школі Черкаської області. Ознайомлено з новітніми методами відбору та кріоконсервації сперми жеребців. Досліджено, що кріоконсервація з використанням попереднього охолодження сперми впродовж 2,5 год та з подальшим швидким замороженням у парах рідкого азоту є ефективним методом для збереження запліднюючої здатності сперми жеребців. Встановлено, якщо дотримуватись цих правил відбору, оцінки, розрахунку, розведення, пакування, кріоконсервації та зберігання сперми високоцінних жеребців, то можна збільшувати кількість банків сперми. Це популяризує дану галузь, дає змогу збільшити кількість господарств з розведення коней та розвивати наукові розробки з біотехнології та репродуктології цих тварин.

Ключові слова: *жеребці, Фантом Маре, штучна вагіна, сперма, розріджувач, пакування, маркування, кріоконсервація*