

The level of alkaline phosphatase's activity at the beginning of the experiment was  $77.30 \pm 1.22$  U/l, and by the 15th day it reached till  $196.50 \pm 3.68$  U/l (increased by 2.5 times,  $p < 0.01$ ). For animals of the control group, the activity of the enzyme was in the range from  $75.63 \pm 1.1$  till  $81.62 \pm 3.7$  U/l throughout the experiment.

After infestation, a significant reduction in glucose in the blood serum of young animals was recorded from  $2.60 \pm 0.05$  till  $2.17 \pm 0.12$  mmol/l by the 15th day after infection (by 17 %,  $p < 0.05$ ). There was also an increase in urea concentration by 52 % and cholesterol by 98 %. Thus, the urea concentration increased from  $4.41 \pm 0.09$  till  $6.72 \pm 0.42$  mmol/l on the 15th day of observation ( $p < 0.05$ ). The concentration of cholesterol increased from  $2.49 \pm 0.09$  till  $4.92 \pm 0.17$  mmol/l ( $p < 0.01$ ). In the blood serum of animals in the control group, it was no significant changes in these indices.

Thus, the parasitization of cattle by the *Oesophagostomum* larvae in the body causes profound changes in the functioning of the host's organism. Thus, a decrease in the number of erythrocytes and hemoglobin leads to a violation of the respiratory function, the development of tissue hypoxia and as a result of intoxication of the body.

The tendency to increase the number of leukocytes is due to the fact that the products of the life of helminths, toxins and products of inflammation are a factor that activates the mechanisms of immunity, aimed at eliminating the antigen. Detected eosinophilia indicates an allergization of the animal's organism by helminths' toxins.

Reducing the glucose concentration in calf serum with oesophagostomosis invasion leads to activation of the gluconeogenesis process – biosynthesis of glucose from substances of non-carbohydrate nature. This leads to a decrease in the concentration of total protein, including albumin, and an increase in the content of the final product of decay of proteins – urea. With this the activation of transferases with the formation of pyruvate and oxalacetate, which is the process of compensatory recovery, is connected.

An increase in the concentration of alkaline phosphatase in serum of animals and an increase in the concentration of cholesterol testifies to the disruption of the liver function of cattle with oesophagostomosis.

It was found that the parasitization of *Oesophagostomum* helminths in the young cattle's body also causes complex disorders of the functions of the systems and organs of the organism.

**Key words:** oesophagostomosis, invasion, cattle, hematology, biochemistry, metabolism.

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#### RELIABLE DIAGNOSIS OF TRICHINOSIS

З огляду на вимоги Міжнародного епізоотичного бюро (МЕБ), Міжнародної комісії з трихinelозу, а також складну ендемічну ситуацію в Україні щодо трихinelозної інвазії, першочерговим завданням є розробка і впровадження в практику ветеринарної медицини новітніх методів діагностики.

Експериментально відтворено низьку (200 личинок на голову), середню (1000 личинок) та високу (20000 личинок) інвазію личинками *Trichinella spiralis* у свиней, що підтверджено їх виявленням в м'язах діафрагми всіх експериментальних тварин після етаназії, за допомогою методів компресоріумної трихinelоскопії і перетравлення проб м'язів в штучному шлунковому соку.

Отримано панель сироваток крові від свиней, експериментально заражених личинками *Trichinella spiralis* в динаміці інвазії, починаючи з 5 до 63 діб після зараження.

За результатами дослідження встановлено, що перетравлення проб м'язів в штучному шлунковому соку з використанням «Набору діагностичного для ідентифікації личинок *Trichinella spiralis* методом перетравлення проб м'язів» є більш чутливим методом післязайної діагностики в порівнянні з методом компресоріумної трихinelоскопії.

Встановлено, що «Універсальний діагностичний набір «Епіскрін АВ» (в комплектації для індивідуальної експрес-діагностики трихinelозу тварин) має високу чутливість і дозволяє виявляти антитіла до *Trichinella spiralis* у свиней на ранній стадії інвазії, починаючи з 8-17 діб після зараження. Методами компресоріумної трихinelоскопії і перетравлення проб м'язів в штучному шлунковому соку неможливо виявити личинок трихinel в зазначені терміни.

За допомогою «Тест-системи діагностичної імуоферментної «*Trichineliso test АВ*», антитіла до *Trichinella spiralis* в сироватці крові експериментально заражених свиней виявлено на 8-22 добу після зараження, що свідчить про досить високий рівень чутливості діагностикума.

«Універсальний діагностичний набір «Епіскрін АВ» дозволяє виявляти антитіла в між'язовій рідині, а також крові з порожнини серця тварин, отриманих після їх забою. Цей набір є експрес тестом, яким можна проводити прижиттєву і/або післязабійну діагностику трихінельозу свиней протягом 15 хв, практично в «польових умовах».

**Ключові слова:** трихінельозна інвазія, діагностика, компресоріумна трихінелоскопія, пепсинізація, ІХА, ІФА.

**Statement of problem.** Trichinosis invasion is spread all over the world, including Ukraine, among commensal and wild animals. On the territory of Ukraine, the majority of cases of human being trichinella contamination are caused by the use of pork. However, for the last 30 years in Europe, the outbreaks of humans trichinosis were caused by the consumption of horse meat, contaminated with *Trichinella* larvae.

Today, the methods of post lethal and vivo diagnostics of trichinosis are well known. The post lethal diagnostics is carried out with the methods of compressorium trichinelloscopia and muscles digestion in artificial gastric juice (AGJ or pepsin method). The vivo diagnostics – by immunological methods *ELISA* and *dot-ELISA*. However, there is still no test-system based on *ELISA* for the trichinosis diagnosis of animals of different species. In the majority of industrialized countries, according to the law, the post slaughter diagnostics is performed with the help of the digestion method of post-slaughter muscles samples in artificial gastric juice and the vivo diagnostics – by immunological methods. But in Ukraine the main diagnostics is the post slaughter by compressorium method, the efficacy of which makes 3–4 larvae for 1 g of muscles. Sensitivity of AGJ method is 1 larva and *ELISA* test is 0.01 larvae for 1 g of muscles.

Thus, considering the requirements of the International epizootic bureau (IEB), International trichinosis commission, the difficult endemic situation of trichinosis invasion in Ukraine, nowadays, the primary task is development and deployment of the modern methods of its diagnostics in practice of veterinary medicine.

**Purpose of our study** – to determine the sensitivity level of serological diagnostic kits, based on Enzyme ImmunoAssay (EIA) and 577-analysis, intended for identification of the antibodies to *Trichinella spiralis* in experimentally infected pigs in comparison with the standard methods of post mortem diagnosis of trichinosis (the compressorium trichinelloscopia and pepsin methods).

**Subject of research** – blood, serum, intra-muscular liquid, muscles of the legs of the diaphragm and hips of pigs infected with larvae *Trichinella spiralis*.

**Research methods** – experimental infection of animals. It have been created 3 research groups from a livestock of pigs of large white breed, 3-month age, average fatness, weighing 20 kg, free from antibodies to *Trichinella spiralis*. It was 3 animals in each group, numbered and placed in individual places. For experimental infection of animals we used mincemeat of rats muscles with *Trichinella spiralis* larvae.

The pigs were given *per os* mincemeat with *Trichinella* larvae in accordance with the following scheme: in the first group for each animal (№2001, №2002, №2003) – 2 g of minced meat that contains 200 *Trichinella spiralis* larvae (low dose); in the second group (№2004, №2005, №2006) – 10 g of minced meat that contains 1000 larvae (medium dose); in the third group (№2007, №2008, №2009) – 200 g of minced meat with 20000 *Trichinella spiralis* larvae (high dose).

Sampling of the stabilized blood and serums from the studied animals we have made before infection (control) and after it, from 5 to 25 day with daily interval, from 25 to 33 day with an interval of three days, from 33 to 63 day with weekly interval. In the case of the detection of antibodies to *Trichinella spiralis*, from each research group one animal was subjected to euthanasia and the remaining pigs were subjected to euthanasia after experimental infection in the 63 days.

From freshly killed carcasses of animals there were chosen for the research: samples of the muscles of the diaphragm and hips with the pepsin methods, using the capsules of modified pepsin («Diagnostic kit for identification of *Trichinella spiralis* larvae with muscles sample digestion method»), and with compressorium trichinelloscopia method; blood samples from the heart (atria and ventricles) with the help of the methods of EIA and 577-analysis, using «Diagnostic enzyme immunoassay test-system «*Trichinelisotest AB*» and «Universal diagnostic kit «*Episkrin AB*»; intra-muscular liquid with the help of 577-analysis.

It was carried out the calculation of quantity of *Trichinella* larvae in muscles samples of the diaphragm under compressorium, held in 48 cuts, and later, after digesting in artificial gastric juice – in 1 g of the studied muscles with the help of light microscope at low magnification (7x8).

Before infection, blood was selected by schedule, starting from 5 to 63 days of experimental invasion:

Дата	19.03	24.03	25.03	26.03	27.03	28.03	29.03	30.03	31.03	1.04
Доба інвазії	0	5	6	7	8	9	10	11	12	13
Дата	2.04	3.04	4.04	5.04	6.04	7.04	8.04	9.04	10.04	11.04
Доба інвазії	14	15	16	17	18	19	20	21	22	23
Дата	12.04	13.04	16.04	19.04	21.04	29.04	6.50	13.05	19.05	
Доба інвазії	24	25	28	31	33	41	48	55	63	

We investigated the stable blood and serum from infected animals in invasion dynamics in order to determine the level of sensitivity of the EIA and 577-analysis methods.

**Results of our study.** According to the results of serological studies of blood samples with the help of 577-analysis method, using «Universal diagnostic kit «*Episkrin AB*», *Trichinella spiralis* antibodies were found in the pig № 2008 in 8 days after infection and in the pigs № 2007, №2009 – in 10 days. The antibodies were detected in the pigs № 2003, № 2005 in 11 days, in 13 days – in the pig № 2001, in 14 days – № 2002. In 17 days the antibodies to *Trichinella spiralis* by means of «Universal diagnostic kit «*Episkrin AB*» were found in all infected animals.

The interpretation of results was conducted by calculating the threshold value (TV). Serum was considered positive, if its optical density (OD) was more 0.250 TV, and negative, if less TV. The antibodies to *Trichinella spiralis* were found in the pig № 2008 with the EIA method in 9 days of experimental invasion, in 12 days – in the pigs № 2003 and № 2009. The pigs № 2007, № 2001, № 2004 were with *Trichinella* antibodies in 13, 15 and 16 days after infection. In the serum of experienced animals № 2002 and № 2006 the antibodies were found in 21 days and for the animal № 2005 – in 22 days of experimental invasion.

Thus, there were identified the antibodies of all studied pigs, subjected to euthanasia, (except for animals № 2002, № 2006, № 2007) with the help of EIA method in 23–24 days after infection to the end of the experiment (up to 63 days after infection).

After the detection of antibodies to *Trichinella spiralis* in all animals of each research group, we conducted the euthanasia of one animal from each group in order to confirm the infection of pigs with *Trichinella larvae*. Thus, in 22 days of experimental *Trichinella spiralis* larvae invasion the pig № 2007 was subjected to euthanasia, in 23 days – the pigs № 2002 and № 2006, in 63 days – the rest of the pigs.

From each of the carcasses of the research animals the samples of muscles of the diaphragm and hips of pigs were selected in order to calculate the quantity of *Trichinella larvae* and to detect the antibodies to *Trichinella spiralis* in intermuscular liquid with compressorium trichinelloscopia method and with digestion in artificial gastric juice. The results of our research of the diaphragm muscles of pigs, experimentally infected by *Trichinella spiralis* larvae, are shown in the table 1.

Table 1 – The results of the research of the diaphragm muscles of pigs, experimentally infected by *Trichinella spiralis* larvae

Group of animals	The infective dose (larvae for one animal)	Individual number of animals	Compressorium trichinelloscopia (larvae /48 cuts)	Larvae digestion for 1g of muscles in artificial gastric juice
1	200	2001	27	115
		2002	59	232
		2003	44	123
2	1000	2004	75	239
		2005	156	388
		2006	130	405
3	20000	2007	1257	4230
		2008	1524	4342
		2009	1337	3939

In the diaphragm muscles, selected from experienced pigs № 2002, № 2006, № 2007 and subjected to euthanasia in 22–23 days after being infected with compressorium trichinelloscopia method, *Trichinella spiralis* larvae had no capsules, haven't been always twisted by a spiral, weren't visible

(Figure 1). That's why it was difficult to make the post mortem trichinosis diagnostics at this stage. However, *Trichinella* larvae were well visible in a microscope, using the method of muscles digestion in artificial gastric juice (Figure 2).

*Trichinella* larvae had a spiral appearance with the capsule created around her in muscles samples, selected from the pigs carcasses in 63 days of experimental invasion, using the compressorium trichinelloscopia method (Figure 3).

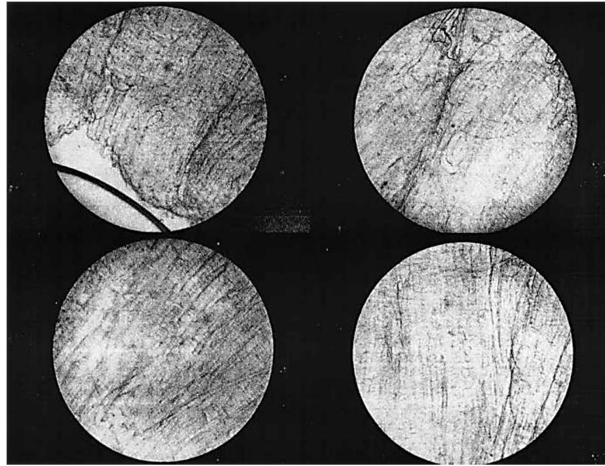


Figure 1. *Trichinella spiralis* larvae in the diaphragm muscles of pigs in 21–22 days of experimental invasion

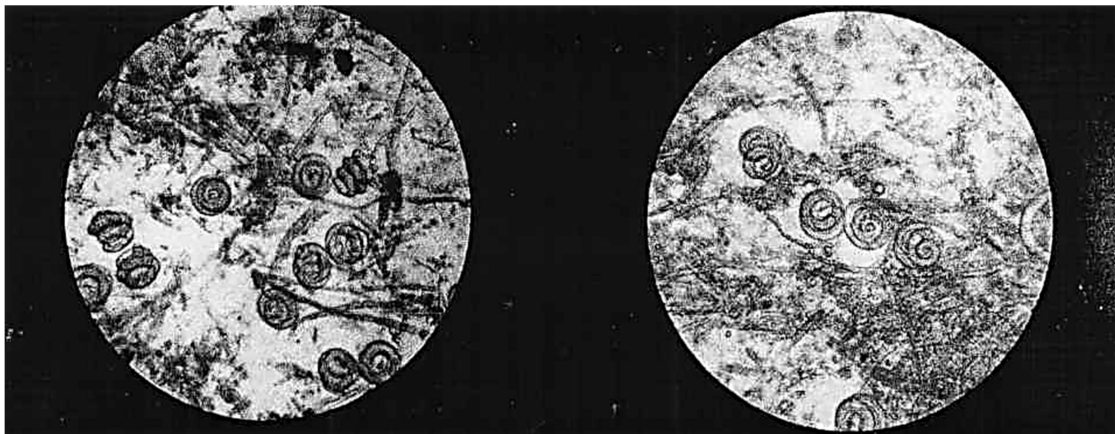


Figure 2. *Trichinella spiralis* larvae in the sludge after digesting the diaphragm muscle samples in artificial gastric juice in 21–22 days of pigs experimental invasion

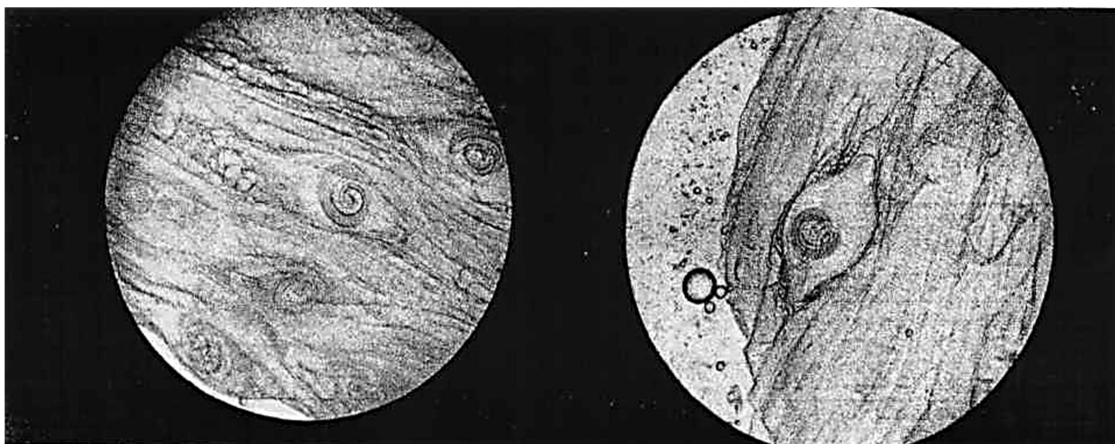


Figure 3. *Trichinella spiralis* larvae in the diaphragm muscles of pigs in 63 days of experimental invasion

In the intra-muscular liquid, in the muscles of the diaphragm and hips, in the blood from the heart, selected from the carcasses of all test animals, there were discovered the antibodies to *Trichinella spiralis* with the help of the EIA method, using «Universal diagnostic kit «Episkrin AB» (table 2).

Table 2 – The results of the research of the diaphragm muscles and hips of pigs, the blood from the heart of experimental animals with the 577-analysis method

Group of animals	The infective dose (larvae for one animal)	Individual number of animals	Night invasion	Detection of antibodies to <i>Trichinella spiralis</i> with the 577-analysis method:					
				in the muscles of the diaphragm		in the muscles of the hips		in the blood from the heart	
				absolute result	result	absolute result	result	absolute result	result
1	200	2001	63	50	+	63	+	76	+
		2002	23	73	+	62	+	74	+
		2003	63	106	+	91	+	117	+
2	1000	2004	63	76	+	74	+	74	+
		2005	63	56	+	62	+	93	+
		2006	23	76	+	79	+	85	+
3	20000	2007	22	61	+	64	+	72	+
		2008	63	71	+	78	+	87	+
		2009	63	96	+	85	+	102	+

In the Table 3 we have presented the outcomes of antibodies identification to *Trichinella spiralis* of the pigs that were infected by *Trichinella* larvae with the EIA and 577-analysis methods. There were also indicated the results of the post mortem diagnostics, the larvae number in the muscle samples of experimental animals, using the compressorium trichinelloscopia method and the method of muscles digestion in artificial gastric juice.

Table 3 – The deadline for the antibodies detection and the results of calculation of quantity of *Trichinella* larvae in the studied pigs

Group of animals	The infective dose (larvae for one animal)	Individual number of animals	The deadline for the antibodies detection (24 hours after infection):		The compressorium trichinelloscopia method (larvae in 48 cuts ~ 0.3–0.4 g)	Digestion of muscles samples in artificial gastric juice (larvae in 1 g of muscles)
			with the help of EIA	577-analysis methods		
1	200	2001	15	13	27	115
		2002	21	14	59	232
		2003	12	11	44	123
2	1000	2004	16	17	75	239
		2005	22	11	156	388
		2006	21	16	130	405
3	20000	2007	13	10	1257	4230
		2008	9	8	1524	4342
		2009	12	10	1337	3939

Summarising the obtained results of serological trichinosis diagnosis of experimentally infected pigs, it can be argued that «Diagnostic enzyme immunoassay test-system «*Trichinelisotest AB*» and «Universal diagnostic kit «*Episkrin AB*» allows to identify the trichinosis invasion of the pigs at an early stage when it is impossible to detect *Trichinella* larvae with the methods of muscles digestion in artificial gastric juice and compressorium trichinelloscopia.

**Conclusions.** 1. It was experimentally reproduced low (200 larvae for one animal), medium (1000 larvae) and high (20000 larvae) invasion of *Trichinella spiralis* larvae of pigs that was confirmed by its identification in the diaphragm muscles of all experimental animals after euthanasia with the help of the methods of muscles digestion in artificial gastric juice and compressorium trichinelloscopia.

2. It was received the serums blood panel from pigs, experimentally infected with *Trichinella spiralis* larvae, in invasion dynamics, going from 5 to 63 days after infection.

3. The research study has found that digestion of muscles samples in artificial gastric juice, by using «Diagnostic set for identification of *Trichinella spiralis* larvae with the help of muscle sample digestion method», is more sensitive method of the post mortem diagnostics in comparison with the compressorium trichinelloscopia method.

4. We discovered that «Universal diagnostic kit «*Episkrin AB*» (individual express-diagnostics of animals' trichinosis) has high sensitivity and can detect the antibodies to *Trichinella spiralis* of pigs at the early stage of invasion, starting from 8–17 days after contamination. It is impossible to detect *Trichinella* larvae in the specified terms, using the methods of compressorium trichinelloscopia and muscles digestion in artificial gastric juice.

5. *Trichinella spiralis* antibodies in serum of experimentally infected pigs have been discovered in 8–22 days after infection with the help of «Diagnostic enzyme immunoassay test-system «*Trichineliso test AB*». It testifies a high sensitivity level of diagnostics.

6. «Universal diagnostic kit «*Episkrin AB*» allows to detect the antibodies in intra-muscular liquid and blood from the animals' heart after slaughter them. This set is an express test, which can be used as trichinosis diagnosis of pigs, during their lives and/or after their slaughter, within 15 minutes.

**Suggestions.** 1. To recommend the use of «Diagnostic enzyme immunoassay test-system «*Trichineliso test AB*» and «Universal diagnostic kit «*Episkrin AB*» for intravital diagnosis of animals' trichinosis.

2. To introduce «Universal diagnostic kit «*Episkrin AB*» in practice of veterinary medicine for the trichinosis diagnosis before the slaughter of pigs and, if it is necessary, for serological studies of animal carcasses after slaughter them.

3. To recommend to experts of veterinary medicine, hunters, individuals to use «Universal diagnostic kit «*Episkrin AB*» for antibodies' identification to *Trichinella spiralis* in the intra-muscular liquid (meat), in the blood of animals' carcasses after their slaughter, as well as bagged during the hunting.

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#### Надежная диагностика трихинеллеза

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Учитывая требования Международного эпизоотического бюро (МЭБ), Международной комиссии по трихинеллезу, а также сложную эндемическую ситуацию в Украине по трихинеллезной инвазии, первоочередной задачей является разработка и внедрение в практику ветеринарной медицины новейших методов диагностики.

Экспериментально воспроизведено низкую (200 личинок на голову), среднюю (1000 личинок) и высокую (2000 личинок) инвазию личинками *Trichinella spiralis* у свиней, что подтверждено их выявлением в мышцах диафрагмы всех экспериментальных животных после эвтаназии, с помощью методов компрессориумной трихинеллоскопии и переваривания проб мышц в искусственном желудочном соке.

Получена панель сывороток крови от свиней, экспериментально зараженных личинками *Trichinella spiralis* в динамике инвазии, начиная с 5 до 63 суток после заражения.

По результатам исследования установлено, что переваривания проб мышц в искусственном желудочном соке с использованием «Набора диагностического для идентификации личинок *Trichinella spiralis* методом переваривания проб мышц» является более чувствительным методом послеубойной диагностики по сравнению с методом компрессориумной трихинеллоскопии.

Установлено, что «Универсальный диагностический набор «Епискрин АВ» (в комплектации для индивидуальной экспресс диагностики трихинеллеза животных) имеет высокую чувствительность и позволяет выявлять антитела к *Trichinella spiralis* у свиней на ранней стадии инвазии, начиная из 8–17 суток после заражения. Методами компрессориумной трихинеллоскопии и переваривания проб мышц в искусственном желудочном соке невозможно обнаружить личинок трихинелл в указанные сроки.

При помощи «Тест-системы диагностической иммуноферментной «*Trichineliso test AB*», антитела к *Trichinella spiralis* в сыворотке крови экспериментально зараженных свиней обнаружены на 8–22 сутки после заражения, что свидетельствует о достаточно высоком уровне чувствительности диагностикума.

«Универсальный диагностический набор «Епискрин АВ» позволяет выявлять антитела в межмышечной жидкости, а также крови из полости сердца животных, полученных после их убоя. Этот набор является экспресс тестом, которым можно проводить прижизненную и/или послеубойную диагностику трихинеллеза свиней в течение 15 мин, практически в «полевых условиях».

**Ключевые слова:** трихинеллезная инвазия, диагностика, компрессориумная трихинеллоскопия, пепсинизация, ИХА, ИФА.

#### Reliable diagnosis of trichinosis

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Thus, considering the requirements of the International epizootic bureau (IEB), International trichinosis commission, the difficult endemic situation of trichinosis invasion in Ukraine, nowadays, the primary task is development and deployment of the modern methods of its diagnostics in practice of veterinary medicine.

«Universal diagnostic kit «*Episkrin AB*» (individual express-diagnostics of animals' trichinosis) has high sensitivity and can detect the antibodies to *Trichinella spiralis* of pigs at the early stage of invasion, starting from 8–17 days after contamination. It is impossible to detect *Trichinella* larvae in the specified terms, using the methods of compressorium trichinelloscopia and muscles digestion in artificial gastric juice.

*Trichinella spiralis* antibodies in serum of experimentally infected pigs have been discovered in 8–22 days after infection with the help of «Diagnostic enzyme immunoassay test-system «*Trichineliso test AB*». It testifies a high sensitivity level of diagnostics.

«Universal diagnostic kit «*Episkrin AB*» allows to detect the antibodies in intra-muscular liquid and blood from the animals' heart after slaughter them. This set is an express test, which can be used as trichinosis diagnosis of pigs, during their lives and/or after their slaughter, within 15 minutes. To recommend the use of «Diagnostic enzyme immunoassay test-system «*Trichineliso test AB*» and «Universal diagnostic kit «*Episkrin AB*» for intravital diagnosis of animals' trichinosis. To introduce «Universal diagnostic kit «*Episkrin AB*» in practice of veterinary medicine for the trichinosis diagnosis before the slaughter of pigs and, if it is necessary, for serological studies of animal carcasses after slaughter them.

**Key words:** trichinosis invasion, diagnostics, the methods of compressorium trichinelloscopia and muscles digestion in artificial gastric juice, the help of EIA and 577-analysis method.

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#### ДИНАМИКА МИКРООРГАНИЗМОВ ЖЕЛУДОЧНО-КИШЕЧНОГО ТРАКТА И ПОКАЗАТЕЛЕЙ КРОВИ ПРИ КОМПЛЕКСНОМ ЛЕЧЕНИИ АССОЦИАТИВНЫХ ПАЗАРИТОЗОВ ТЕЛЯТ

Изучено действие на состав микрофлоры толстого кишечника крупного рогатого скота, а также на биохимические и гематологические показатели крови пробиотика и растительного пребиотика, применяемые в комплексном лечении при дисбиозах, вызванных ассоциативными паразитами. У больных телят было установлено нарушение обменных процессов, что свидетельствует о развитии патологического процесса. В результате применения комплексного лечения наблюдалось восстановление обмена веществ. Наилучший эффект показало применение противопаразитарного препарата в сочетании с растительным пребиотиком и пробиотиком. Данные препараты улучшают состав микроорганизмов и стимулируют развитие собственной нормофлоры.

**Ключевые слова:** ассоциация, дисбактериоз, крупный рогатый скот, микрофлора, паразит, показатели крови, растительный пребиотик, пробиотик, толстый кишечник.