Improvement of lifetime and post-mortem diagnostics of trichinellosis in animals of the Suidae family (comparative study)

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Abstract. Trichinosis is one of the most dangerous helminthozoonoses. Methods of compressor trichinelloscopy and peptolysis of animal muscle tissue in artificial gastric juice used for postmortem diagnosis of trichinosis are reliable enough to identify sources of infection and prevent the development of helminthiasis in humans. In addition to these methods, indirect solid-phase enzyme immunoassay (ELISA) and crystalloscopy were used. Meat juice samples obtained from domestic pigs from various regions of the Russian Federation and wild boars were used as the tested biological material. The formulation of ELISA and crystalloscopy, accounting and interpretation of the results obtained were carried out according to the classical method: the result was considered positive, exceeding the negative control by 0.08 units or more in optical density. The specificity of ELISA in all animals with heterologous invasion was 98%, hydrosol-hydrogel reactions - 80% and crystalloscopy - 75%. These methods can be used for testing for trichinosis, but the examination should be comprehensive: immunological and crystallographic methods should be confirmed by compressor trichinelloscopy and peptolysis using artificial gastric juice. Currently, in the system of veterinary and sanitary measures for trichinosis, there is a change in the structure of the sources of invasion. The probability of human infection with hunting products is higher than when eating pork obtained from meat processing enterprises. Therefore, field trichinelloscopic control in the field (CT, HH etc.) occupies a leading place in diagnostics. Thus, the development of portable equipment for veterinary examination and the training of hunters in basic trichinelloscopy skills, as well as the improvement of the formulation and accounting of crystalloscopy and HH are becoming extremely relevant.

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1 Introduction

Trichinosis is one of the most dangerous helminthiasis common in vertebrates. This zoonosis has great medical, veterinary and social significance. Despite the fact that its causative agent (*Trichinella spiralis*) was discovered back in 1835, the issues of diagnosis and prevention of trichinosis in representatives of the family Suidae (order Artiodactyla) are still relevant. It has been proved that all the main species of trichinella parasitize domestic and wild pigs: *Trichinella spiralis* Owen, 1835, *Trichinella pseudospiralis* Garkavi, 1972, less often *Trichinella nativa*. The development cycle is completely completed in the body of a pig or boar (the intestinal stage lasts up to 3-9 weeks, and the muscular stage lasts up to 20 months or more). Meat becomes deadly to humans, and in accordance with the provisions of regulatory documents of the Russian Federation (Sanitary Norms and Rules of the Russian Federation, 2014) it must be disposed of (only internal fat, bacon and skin can be used). Postmortem diagnostics has been mandatory at all processing plants since 1923. Currently, the main methods of diagnosis of this disease, regulated by regulatory documents of the Russian Federation, are postmortem methods and enzyme immunoassay. Postmortem methods include compressor trichinelloscopy of 24 sections (CT) and the peptolysis (PS) method, in which the sediment is examined after digestion in artificial gastric juice. These methods are regulated for all meat processing enterprises in the study of meat from domestic pigs (*Sus domesticus*, or *Sus scrofa domesticus*). Despite the development of portable trichinelloscopes, this procedure is still difficult to implement when studying wild boars in the wild, although trichinosis is widespread in wild animals, including wild boars (*Sus scrofa*) [1, 2, 3]. Given that wild boar extraction is usually carried out far from populated areas, therefore, sanitary work among the population and the possibility of checking meat before consumption by hunters and their family members are of great preventive importance. Another important aspect is the overdiagnosis of trichinosis in wild boars. Even qualified veterinary specialists often, guided only by computed tomography, identify sarcocysts as larvae of trichinella (*S. miescheriana*), encapsulated Alaria, Agamospirura, etc. And, thus, meat is destroyed, which, with appropriate processing, can be used for food. Therefore, an equally important aspect is the possibility of preserving samples of the studied muscles to confirm or refute the diagnosis. In this case, if questionable results are obtained (the presence of capsules in muscle tissue without larvae or with damaged or destroyed meat), meat with suspected trichinosis must be frozen until the final diagnosis is confirmed, and then follow the instructions of regulatory documents accordingly (Sanitary Norms and Rules of the Russian Federation, 2014). Veterinary medicine in the Russian Federation has a sufficient arsenal of equipment for trichinelloscopy, including portable devices (TP-1 and TP-2) with an autonomous power source for field trichinelloscopy. To confirm the diagnosis, it is necessary to conduct postmortem examinations by classical trichinelloscopy and PS-based methods using AVT-series devices, in addition, other methods commonly used for lifetime diagnosis can be used [4]. The reports of many authors on the possibility of using methods developed for in vivo diagnosis and for postmortem diagnosis [5, 6, 7, 8].

The first diagnostic reactions for the diagnosis of trichinosis were precipitation reactions on live trichinella invitro, then somatic antigens were used, and later excretory-secretory or metabolic antigens (products of isolation of Trichinella larvae). Until the nutrient media for culturing larvae and producing excretory-secretory antigens were improved, the sensitivity was 93% and the specificity was 92% (Mauss E, 1990). Further improvement of the methods of purification of media and the excretory-secretory antigens themselves and the development of optimal parameters for the cultivation of Trichinella made it possible to increase these parameters [9, 10, 11, 12, 13, 14]. Further, various agglutination reactions were studied, but ELISA became the most effective (Uspensky A.V., 2019) [2, 3, 15, 16, 17]. Currently, in mass epidemiological and epizootic studies, an indirect variant of enzyme
immunoassay (ELISA) and its numerous modifications (a-ELISA, dot-ELISA) are used, in addition to immunological reactions, studies of the level of crystallogenic activity of the biological environment (crystalloscopy) in trichinosis of domestic and wild animals, studies of representatives of the Suidae family have been conducted. This method is based on the fact that parasites affect the composition of biological fluids of the organism, which lead to transformation of its physical and chemical properties. It induces the specific changes of dehydration structurization of different biofluids in trichinellosis and can be visualized by crystalloscopic and tezigraphic test. We stated that crystalloscopic picture of blood serum and urine is transformed specifically (phenomenon of parasite-associated crystallogenesis). There are also similar immunochemical methods using hydrogel hydrogels (HH) based on iron preparations based on a change in the color of the substrate. Considering the above, it is important to analyze the available methods for diagnosing trichinosis and continue to develop an express technique that could be used as a quick and simple method, easily carried out in the field, providing a sufficient degree of accuracy and the possibility of wide application without investing significant material resources and a large amount of time. A method for preserving muscle tissue for subsequent clarifying trichinelloscopy (CT and PS) [7, 8, 9] Taking into account the above, the purpose of this work is to compare the effectiveness of postmortem diagnostic methods, including those developed for the lifetime diagnosis of trichinosis, such as the study of meat juice for PS and crystallographic analysis and HH of meat juice.

2 Materials and methods

The method of indirect solid-phase ELISA was used for diagnostic purposes. Samples of meat juice and whey obtained from domestic pigs from various regions of the Russian Federation, as well as wild boars obtained as a result of scientific shooting, were used as the tested biological material. ELISA was used, and the results were taken into account in accordance with the classical method: the result was considered positive, exceeding the negative control by 0.08 units or more in optical density. The effectiveness of ELISA in trichinosis was calculated for more than 100 blood serum samples, including those from experimentally infected T. spiralis and E. granulosus, naturally infected C. tenuicolis (5) and O. dentatum (2), and clinically healthy animals (90). In addition to ELISA, in order to complement the diagnostic algorithm, the results of crystalloscopy of the blood serum of healthy pigs and pigs experimentally infected with T. spiralis (the dose of the pathogen is 1000 larvae) were evaluated, a bio-liquid was obtained and a study was conducted using the method of Martusevich et al. (2013), and the effectiveness of immunochemical reactions using hydrogel gels (HH) was studied. All studies using ELISA, mercury column and teziocrystalloscopy in clinically healthy pigs and wild boars were accompanied by post-slaughter diagnostics to detect trichinella by CT, and the PS-method was used to confirm the diagnosis. Freezing, solutions of formalin (1%), propyl alcohol (70%) and sodium azide (0.3%) were used to preserve muscle tissue. [12].

3 Results and discussion

The specificity of ELISA in all animals with heterologous invasion was 98%. Animals with echinococcosis (2.54%) were false positive due to the presence of antigenic determinants common to T. spiralis and E. granulosus. The sensitivity was 100% in both the group of naturally infected and the group of experimentally infected animals. In the study by crystalloscopic methods (CM), the obtained set of values and the samples themselves were compared with samples of their own and initiated crystallization and the namesake
crystalloscopic "trichinosis pattern". If at least 6 values of the teziocrystalloscopy parameters coincided with the "pattern", the presence of trichinosis was recorded. As a result, for all meat juice samples from infected animals, the crystalloscopic pattern corresponded to trichinosis confirmed in PS. The analysis of the own and initiated crystallization of meat juice and the use of hydrogel hydrogels also made it possible to confirm differences in crystalloscopic samples in healthy and trichinosis-infected pigs and wild boars, as well as to establish a sufficient level of criteria to achieve sensitivity of 90%. However, the specificity of these tests could not be increased by more than 80% for hydrogels and 78% for crystalloscopy. The disadvantage of the test is not only its relatively low specificity and sensitivity, but also the lack of the possibility of its mechanization and labor intensity. Although crystallographic methods and HH can be recommended for primary screening and selection of questionable samples with further investigation in ELISA [1, 4, 5, 8]. Of the above methods, HH is the most easy to set up, and the method does not require additional equipment. It can be used to diagnose both meat juice and blood serum. But the priority method for initial scanning of serum or meat samples may be HH. However, the main confirmatory method should be the peptolysis method (PS).

Thus, of the entire arsenal of diagnostic methods, field trichinelloscopes of the TP-1,2 type are the most effective and convenient, allowing for an operational examination of carcasses and meat products in difficult production conditions. The advantage of these devices is their high diagnostic efficiency, ease of assembly and autonomy in use. However, in order to avoid hypo- and overdiagnosis of trichinosis, it is necessary to conduct courses for hunters to identify trichinella larvae. In doubtful cases, it is necessary to provide material from shot animals to a veterinary specialist [1, 6, 9].

During CT and PS studies of formalin (1%), propyl alcohol (70%) and sodium azide (0.3%) solutions preserved in solutions or frozen meat, it was found that freezing and storage in 0.03% sodium azide solution is the least aggressive for the structure of the myosimplast and liver capsule. Trichinella. Thus, these methods of preserving samples can be used in doubtful cases to establish a definitive diagnosis by veterinary specialists.

Table 1. Comparison of the effectiveness of methods of postmortem diagnosis of trichinosis.

<table>
<thead>
<tr>
<th>Performance indicators</th>
<th>ELISA</th>
<th>Crystalloscopy</th>
<th>HH</th>
<th>CT 72 slides</th>
<th>PS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>100</td>
<td>90</td>
<td>90</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>98</td>
<td>78</td>
<td>80</td>
<td>90</td>
<td>100</td>
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4 Conclusions

Currently, in the system of veterinary and sanitary measures for trichinosis, there is a change in the structure of the sources of invasion. The probability of human infection with hunting products is higher than when eating pork obtained from meat processing enterprises. Therefore, field trichinelloscopic control in the field (CT, HH etc.) occupies a leading place in diagnostics. Thus, the development of portable equipment for veterinary examination and the training of hunters in basic trichinelloscopic skills, as well as the improvement of the formulation and accounting of crystalloscopy and HH are becoming extremely relevant. [6-8, 18-21]

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