Volume 5, issue 1, 2016 • e-ISSN: 1857-8187 • p-ISSN: 1857-8179

Review Article

Investigation on Trichinellosis of pigs in Saranda District

Healthcare

Keywords: Trichinella, pig, diagnosis, ELISA, Saranda.

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Abstract

Trichinellosis caused by the Nematode Trichinella is azoonotic disease which occurs worldwide and affects abroad range of different species including mammals, birds and amphibians. Trichinella spiralis is found in production animals (pigs, horses) in temperate climate zones and can also be found in animals in close contact with these production animals (e.g. dogs, cats, rats). The PrioCHECK® Trichinella Ab is a reliable and fast diagnostic test for detection of antibodies against Trichinella in porcine serum and meat juice samples, and can be used for monitoring and surveillance purposes.

Introduction

Trichinellosis is a parasitic infection caused by the larval and adult forms of some parasitic nematode Trichinella belonging gender. An important characteristic of this infection is that it is zoonotic and infectious larvae are meat borne (usually pork, but ever more also other animal meat). Human infection occurs by ingestion of cystic *Trichinella* larvae found in muscle tissue of domestic or wild animals. These parasites are spread in the wild in all continents except Antarctica, and in many countries in domestic pigs (Pozio and Murrell, 2006). Due to the political and economic changes, it has recently been observed increase in prevalence and incidence of this parasitic infection in many countries of Eastern Europe (8, 19, 24). Such increases are mainly related with an inefficient veterinary service on the control of meat products originating from animals receptive. This presents a serious problem for the pork trade within the European Union countries and the export of pork outside these countries. As a result of the European emergency problem, some member states of the European Union and associated countries not members of the European Union, have implemented a program to monitor the *Trichinellosis* in pigs, horses, wild boar and other species of wildlife (32).The European Commission has implemented a new regulation, no. 2075/2005, which sets specific rules for official controls of *Trichinella* in meat to improve the safety of food for European consumers. Animals can be tested for the presence of anti-*Trichinella* antibodies in serum or meat juice, ante mortem or postmortem during necropsy (108). According to the International Commission for *Trichinellosis*, indirect methods such as the detection of *Trichinella* antibodies in domestic and wild animals are not recommended as a substitute method for inspecting individual meat carcasses (49). However, serology for diagnosis of *Trichinella* is considered to be appropriate for surveillance and epidemiological investigations of domestic and wild animals (51). Based on the above consideration we have realized the study entitled "Investigation on *Trichinellosis* of pigs in Saranda District".

Material and methods

The study was carried in 90 pigs of several farms in the area of Saranda. Blood was collected from the jugular veins of these animals by sterile containers. Subsequently the blood was left at room temperature for 24 hours and serum was taken from him. The serum obtained in this way was collected in special containers and was stored frozen at -40 ° C temperature. To conduct the study was used PrioCHECK® *Trichinella* Ab, an indirect ELISA for the detection of antibodies against *Trichiella spp*. in blood and meat juice of pigs and the reaction was conducted by the following procedure:

1. Preparation of samples

- Was reconstituted the control sample by adding 150 µl of demineralized water (delivered with the kit).
- Added 10µl of the positive control to wells A1 and B1 of the dummy plate with 96 wells.
- Added 10 µl of Weak Positive Control to wells C1 and D1 of the dummy Plate.
- Added 10 µl of Negative Control to wells E1 and F1 of the Dummy Plate.
- Added 10 µl of serum samples to the remaining wells of the Dummy Plate,
- Added 90 µl of Sample Diluent to each well of the Dummy Plate and mix by pipetting up and down 5 times.
- Added 80 µl of Sample Diluent to each well of the Test Plate.
- Was Transferred 20 µl of the diluted samples and controls from the Dummy Plate to the Test Plate and was mixed by pipetting up and down 5 times.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Positive Control	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35	Sample 43	Sample 51	Sample 59	Sample 67	Sample 75	Sample 83
B	Positive Control	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36	Sample 44	Sample 52	Sample 60	Sample 68	Sample 76	Sample 84
C	Weak Positive Control	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37	Sample 45	Sample 53	Sample 61	Sample 69	Sample 77	Sample 85
D	Weak Positive Control	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38	Sample 46	Sample 54	Sample 62	Sample 70	Sample 78	Sample 86
E	Negative Control	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39	Sample 47	Sample 55	Sample 63	Sample 71	Sample 79	Sample 87
F	Negative Control	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40	Sample 48	Sample 56	Sample 64	Sample 72	Sample 80	Sample 88
G	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33	Sample 41	Sample 49	Sample 57	Sample 65	Sample 73	Sample 81	Sample 89
H	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34	Sample 42	Sample 50	Sample 58	Sample 66	Sample 74	Sample 82	Sample 90

Figure 1. The scheme of establishing positive, negative samples and those that will be tested at the plate with 96 wells of ELISA kit.

Sample Incubation

- Test plates, organized as above, were incubated for 30±1 minute at room temperature (22 ± 3°C).
- After incubation the plates was washed with working solution according to instruction of the test.

Conjugate Incubation

- Was Diluted the needed amount of Conjugate (30x) 30 fold in Conjugate Diluent
- It added 100 µl of the diluted conjugate for each well of test plate.
- After adding the conjugate, the plate was incubated for 30±1 minute at room temperature (22±3°C).
- After the incubation the plate was washed with working solution for 4 times.

Detection

- It added 100 µl chromogen substrate (TMB) to each well in a test plate.
- Test plates was incubated for 15 ± 1 minute at 22 ± 3°C.
- After incubation was added 100µl of the stop solution to each well of the plate test
- Was shaken the test plate shortly (5-10 s.) either on an orbital shaker (~300 rpm) or manually on the working bench.
- Was read the Test Plate in the ELISA reader at 450 nm within 15 minutes.

Result Interpretation

- Calculation of results
- It was realized based on the following formula:

$$\frac{OD_{450}\ \text{sample}}{OD_{450}\ \text{positve sample}} \times 100 = x\%\ \text{positivity}$$

- The mean OD450 of the Positive Controls must be >1.0
- The mean percentage of positivity (PP) of the Weak Positive Controls must be > 35%
- The mean OD450 of the Negative Controls must be <0.2

Results

The results obtained after reading performance of ELISA test on blood serum samples from pigs collected from Saranda area, expressed in optical density OD presented in the tables below:

Table 1. Optical density values obtained after reading multiscan with 450nm filter

1.473	0.032	0.033	0.031	0.044	0.034	0.026	0.033	0.039	0.061	0.043	0.036
1.394	0.033	0.039	0.03	0.041	0.03	0.038	0.031	0.032	0.031	0.034	0.034
0.457	0.041	0.041	0.03	0.032	0.031	0.034	0.033	0.033	0.033	0.03	0.034
0.478	0.038	0.034	0.034	0.033	0.029	0.075	0.057	0.032	0.028	0.036	0.031
0.042	0.043	0.035	0.032	0.031	0.034	0.044	0.035	0.033	0.028	0.039	0.031
0.041	0.042	0.046	0.032	0.031	0.035	0.044	0.038	0.041	0.04	0.039	0.031
0.081	0.131	0.041	0.033	0.042	0.037	0.036	0.059	0.042	0.035	0.04	0.041
0.043	0.042	0.041	0.041	0.045	0.043	0.043	0.042	0.043	0.037	0.037	0.041

Table 2. OD positivity percentages calculated according to the above formula

102.7555	1.474654	1.520737	1.428571	2.02765	1.56682	1.198157	1.520737	1.797235	2.81106	1.981567	1.658986
97.24451	1.520737	1.797235	1.382488	1.889401	1.382488	1.751152	1.428571	1.474654	1.428571	1.56682	1.56682
31.88001	1.889401	1.889401	1.382488	1.474654	1.428571	1.56682	1.520737	1.520737	1.520737	1.382488	1.56682
33.34496	1.751152	1.56682	1.56682	1.520737	1.336406	3.456221	2.626728	1.474654	1.290323	1.658986	1.428571
2.929892	1.981567	1.612903	1.474654	1.428571	1.56682	2.02765	1.612903	1.520737	1.290323	1.797235	1.428571
2.860133	1.935484	2.119816	1.474654	1.428571	1.612903	2.02765	1.751152	1.889401	1.843318	1.797235	1.428571
3.732719	6.036866	1.843318	1.520737	1.935484	1.705069	1.658986	2.718894	1.935484	1.612903	1.843318	1.889401
1.981567	1.935484	1.889401	1.889401	2.073733	1.981567	1.981567	1.935484	1.981567	1.705069	1.705069	1.889401

If we observe carefully reading the results obtained after the reaction in the ELISA reader will note that the values of the OD (optical density) are too low. This fact is confirmed after the calculation according to the above formula where the percentage of positivity goes 1.382488, which is the lowest percentage (regards serum placed in well B 4), to the values of the percentage of positivity (PP), 6.036866, which regards serum located in well in G2. In conclusion we can say that none of the sera tested was not positive for trichinella infection. However, as mentioned earlier, we cannot declare the area free from Trichinella infection only ELISA tests.

Although Enzyme-linked immunosorbent assay (ELISA) is the method most commonly used for the detection of infection by Trichinella, mainly because of the sensitivity of the method, which allows detection from 1 larva per 100g tissue muscle and sensitivity of ELISA varies between 93.1 and 99.2%, while specificity ranged from 90.6 to 99.4%, after examination of samples of serum of pigs which originate from farms free Trichinella-free, it cannot be used as confirmatory test for the presence or not of trichinella infection. The European Commission, through the new regulation, no. 2075/2005, sets specific rules for official controls for the presence of trichinella in meat, to improve the safety of food for European consumers. Thus, in addition to controls by indirect methods, each carcass of pigs slaughtered, used for public consumption, must is subject of

direct examination for the presence or not of trichinella (Regulation no. 2075/2005 of the European Commission). Based on rules established, indirect ELISA can be used as a screening method in the sera-surveillance strategies to give a general picture of this food borne zoonotic parasitosis.

Conclusions

After analyzing the ELISA test of 90 samples of serum swine they all resulted negative for trichinella infection. Indirect ELISA is a quick method with a sensitivity ranging between 93.1 and 99.2% and a specificity ranging from 90.6 to 99.4%. Through it can be detected 1 larva per 100 g of muscle tissue. Although high-value diagnostic ELISA cannot be used as a confirmatory test to determine the areas free from *Trichinella*. It can only be used as a screening test in the sera-surveillance program to clarify the epidemiological situation of *Trichinella* infection. Based on the Regulation no. 2075/2005 of the European Commission, each carcass of pigs slaughtered for human consumption should be checked for the presence or not of Trichinella in muscular tissues.

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