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Review Article

The Use of ELISA for Detection of Antibodies Against *Trichinella* Spp., in the Serum of Pigs to Kurbin District

Healthcare

Keywords: Trichinella, pig, serology, diagnosis, ELISA, Kurbin.

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Abstract

Trichinellosis, an infection caused by the Trichinellaspp has been known to occur worldwide affecting almost all species of animals including man. It is an emerging or re- emerging disease in developed and developing countries. This survey was conducted to investigate the prevalence of trichinellosis in domestic pigs slaughtered in kurbin area, Albania. Three hundred sixtypig sera were collected at slaughter in an abattoir in kurbin . The sera samples were stored frozen at the parasitology research laboratory of the Department of Veterinary Public Health, Agricultural University of Tirana. Forserologicaldiagnosiswas useda indirect ELISA,PrioCHECK® Trichinella Ab. From the study resulted positive threepigs or 0.83% of serumtested.

Introduction

Trichinellosis is a parasitic disease that in the past has not always been recognised for its importance. However, it is becoming increasingly clear that greater priority should be given to this zoonosis because ofits health and economic impact, particularly in resource-poor countries Enzyme-linked immunosorbent assay (ELISA) is the most commonly used method for the detection of *Trichinella*infection due mainly to the methodical sensitivity that allows the detection of as low as 1 larva per 100 g of muscle tissue (Office International des Epizooties 2004).

Thus, a large series of experimental and/or field studies has been carried out using pig serum and meat juice samples (Gamble H R and I V Patrascu1996,Gamble H. R et al, 1996,Jakob H P 1994, Nockler K et al,Van der Leek et al 1992). The specificity of ELISA became greatly improved by utilizing metabolic E/S antigens released from *Trichinella*muscle larvae and generated upon in vitro maintenance.The sensitivity of ELISA ranged from between 93.1 and 99.2%, whereas the specificity varied from 90.6 to 99.4% after examination of serum samples of pigs which originated from *Trichinella*-free farms (Murrell K D 1986).

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 (Blaga Ret al 2007, Cuperlovic K et al 2005, Djordjevic M et al 2004).

Albania, after the economic and political changes, it is the first time that used ELISA for serological diagnosis of Trichinella in serum of pigs. The study was conducted in pigs Kurbin area, where predominate extensive breeding farms of swine. Blood was collected at slaughter and serum was taken from him. Data on animals marked in the respective tables.

Material and Methods

The study was conducted in 270 pigs of Kurbin area, where predominate extensive breeding farms of swine. Blood was collected at slaughter and serum was taken from him. Blood was collected from the jugular venis 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 indirekt ELISA for the detection of antibodies against *Trichiella spp*. in blood of pigs and the reaction was conducted by the following procedure:

Preparation of samples

- Was reconstituted the control sample by adding 150 µl of demineralized water (delivered with the kit).
- Added10µl ofthepositivecontroltowellsA1andB1 of thedummyplatewith96wells.
- 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.

Sample Incubation

- Testplates, organizedas above, wereincubatedfor 30±1minuteat room temperature(22 ±3°C).
- Afterincubationtheplateswas washed with working solutionaccording toinstruction of thetest

Conjugate Incubation

- Was Diluted the needed amount of Conjugate (30x) 30fold in Conjugate Diluent
- Itadded100µl ofthediluted conjugateforeachwellof testplate.
- After adding theconjugate, the platewasincubatedfor30±1minuteat room temperature(22±3°C).
- After the incubation the plate waswashed with workingsolutionfor4times.

Detection

- It added 100 µl chromogen substrate (TMB) to each well in atest plate.
- Testplates were incubated for 15±1 minutes at 22±3°C.
- After incubation was added 100µl of the stop solution to each well of the plate test
- It 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

- Calculationof results
- It was realizedbased onthe followingformula:

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

- 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 obtained above or equal the cut-off of 15 PP are considered positive.
- Results obtained below the cut-off of 15 PP are negative.

Results and Discussion

The results obtained after reading performance of ELISA test on blood serums amples from pigs collected from Kurbin area, expressed in percentage of positivity (PP) presented in the following charts:

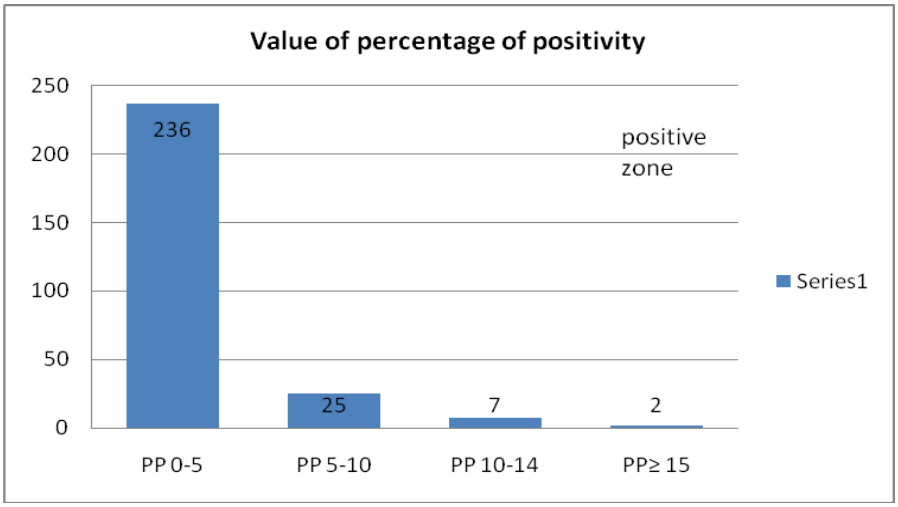

Chart 1. Value of percentage of positivity obtainedafter reading inmultiscanexpressed in number of heads pigs

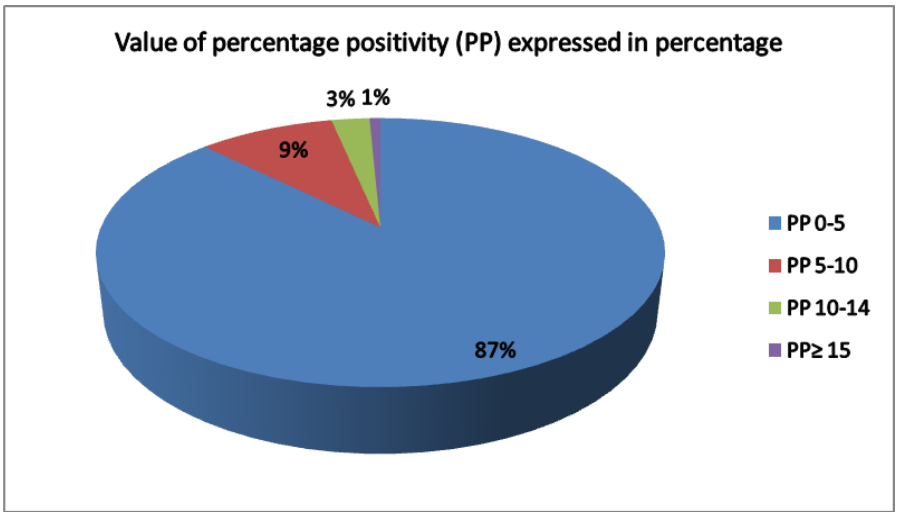

Chart 2. Value of percentage of positivity obtained after reading in multiscan expressed in percentage of heads pigs

As noted by the graphics, in general the percentage values, after the reading in the multiscan are very low. However, have tested positive for the infection of *Trichinella*2 headings or 0.77 percent of the heads tested. This result corresponds with studies of some authors (Wang and Cui 2001; Liu and Boiureau, 2002).

However, 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).

Conclusions

From 270 swine seratested by ELISA have resulted pozitive 2 sera or 0.77% of thoshe tested. 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.

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