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Development and validation of an Enzyme-Linked Immunosorbent Assay for the detection of antibodies against *Trichinella* spp. in domestic pigs in Argentina

Eliana Riva¹, Pedro Steffan¹, Fabián Amanto¹, Agustín Ávila², Gisele Bernat¹, <u>Silvina Fernández¹</u>, Mariana Fuentes¹, Silvia Estein¹

¹Centro de Investigación Veterinaria de Tandil (CONICET-FCV-CICPBA), UNCPBA, Tandil, Argentina. ²Salud Ambiental, Ministerio de Salud de Río Negro, Viedma, Argentina

Abstract

Trichinellosis is endemic in Argentina, with human outbreaks having mainly domestic pigs as focalised sources of infection. Enzyme-Linked Immunosorbent Assay (ELISA), based on excretory/secretory T. spiralis muscle larvae antigens (E/S ML) is the most recommended technique for epidemiological surveillance to detect anti-Trichinella antibodies in domestic pigs. Available international ELISA kits are expensive and need to be validated in each country. Thus, the aim of the present study was to evaluate the performance of an in-house indirect ELISA based on E/S ML antigen. The artificial digestion (AD) was used as reference technique. A total of 343 swine serum samples were used to define sensitivity, specificity, accuracy and repeatability of the ELISA. Sera tested were from 91 outdoor pigs associated with local outbreaks, 12 of them diagnosed as positive by AD, and 242 serum samples from intensiverearing farms with strict hygienic conditions and negative results by AD. Sera from 2 pigs experimentally infected with 10,000 T. spiralis ML (bled at five different days post infection) were also included. E/S ML antigen was diluted at 5 μ g/ml in coating buffer, samples were diluted 1/100 in wash buffer and peroxidase conjugated rabbit anti-pig IgG was used at 1/2500 dilution (Sigma). Reaction was developed using ortho-phenylene-diamine diluted in a citric acid buffer 0.07% H₂O₂. After 15 minutes, reaction was stopped with H₂SO₄ 2.5N. The cut-off point was calculated based on the OD values of 5 negative controls (NC) using the formula: Mean NC ± 3 SD. The diagnostic sensitivity and specificity of the ELISA were 95.5% and 98.4%, respectively. The accuracy was 98.2%. The inter-assay repeatability (4 plates) calculated for 1 positive and 4 negative controls was under 20%. The performance of this ELISA suggests that it could be used as a reliable and practical tool for seroepidemiological studies in Argentina.