IMMUNODIAGNOSIS OF TRICHINELLA INFECTION IN THE HORSE

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Summary:
From 1998 to 2000, 5,267 horse sera were collected from several Trichinella regions in Romania. Sera were initially screened in laboratories in Romania, Serbia and Italy with an EUSA and a Western blot (Wb) using an excretory/secretory (ES) antigen and several conjugates [protein A, protein G, and sheep or goat anti-horse]. Differences in serology results were obtained among the different conjugates and also between EUSA and Wb. Depending on the test used, specific antibodies were found at a prevalence rate of 3-6% of horses. Serum samples classified as positive were tested again by EUSA using a synthetic tyvelose glycan-BSA antigen, in Italy. All serum samples tested using this antigen were negative; in contrast, serum samples from experimentally infected horses were positive with the glycan antigen. The negative results obtained with the glycan antigen are consistent with the low prevalence of horse trichinellosis reported in the literature. Based on these results, further studies are needed to validate immunodiagnostic tests to detect Trichinella infection in horses.

KEY WORDS: horse, trichinellosis, epidemiology, serology, Romania.

Trichinellosis acquired from eating horsemeat has represented a serious health problem since 1975. In the last 26 years, more than 3,300 persons acquired Trichinella infection from eating raw or undercooked horsemeat in France and Italy (Boireau et al., 2000). In Romania, horses are not slaughtered for human consumption, but some are exported to the European Union where horsemeat is consumed. In the last 10 years, the prevalence of Trichinella infection in pigs of Romania increased dramatically up to 5% (Olteanu, 1997); similar increases have been reported in other Eastern European countries (Murrell & Pozio, 2000). At the same time, the number of horses infected with Trichinella, originating from this geographical area, increased considerably, but the actual prevalence of Trichinella infection in this domestic animal is unknown. In 1996, a Trichinella infected horse imported from Romania was detected at a slaughterhouse in Italy (Pozio et al., 1997).

The aim of the present study was to determine the serological prevalence of Trichinella infection in Romanian horses.

MATERIALS AND METHODS

A total of 5,267 animals from the Alba, Cluj, Covasna, Galati, Gorj, Dolj and Timis districts of Romania were included in this study. Serum samples were collected in June 1998 (130 samples) and between November 1999 and June 2000 (5,137 samples). Blood was allowed to clot in non-coated tubes and serum was recovered and stored at -20°C until used.

An excretory/secretory (ES) antigen from Trichinella spiralis muscle larvae (Gamble et al., 1988) was used for both ELISA and Western blot (Wb) analyses in the initial screening of serum samples. Serum samples were diluted 1:50. A serum sample from an experimentally infected horse was used as a positive control and a pool of serum samples from horses slaughtered at a Belgrade slaughterhouse (proved to be Trichinella-free by peptic digestion) were used as negative controls. As a control reaction, monoclonal antibody 7C2C5 (Gamble & Graham, 1984) was used in Wb to recognize the Trichinella specific epitope present on 45, 49, 53 kDa ES antigens. Protein A-HRPO was applied as a conjugate in the ELISA (“TS Poly” ELISA test, IVD Research Laboratories, USA) in the first screening of all serum samples in Romania. Two other conjugates (sheep anti-horse-HRPO and protein G-HRPO, “Trichinella ELISA Test – Horse”, INEP, Belgrade, Yu) were used to screen 507 serum samples at INEP (Belgrade, Yu). All ELISA tests were performed according to the manufacturer's instructions. At INEP, the Wb analysis using ES antigen was carried out on

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44 serum samples, which were positive by ELISA, with three conjugates: anti-horse-HRPO, protein A-HRPO, or protein G-HRPO (the last two from ICN, Biomedical Inc., USA). At ISS (Rome, Italy), only 74 serum samples, which were positive or weakly positive by ELISA in the first screening in Romania, were studied by ELISA using an ES antigen and a goat-anti-horse (Kirkgaard & Parry Laboratories, MA, USA) second antibody and by Wb using an ES antigen and two conjugates (anti-horse-HRPO and protein A-HRPO, Biorad, USA). At ISS, serum samples that were positive by both ELISA and Wb, were examined by ELISA using a synthetic tyvelose glycan-BSA antigen (Heska Co., Fort Collins, CO, USA) and a goat-anti-horse second antibody.

RESULTS

In Romania, of 5,267 serum samples examined by ELISA, 311 (5.9 %) were positive using the protein A-HRPO as a conjugate. In Belgrade, of 507 serum samples examined by ELISA, 67 (13.2 %) were positive using protein A-HRPO, 26 (5.1 %) using protein G-HRPO, and 18 (3.5 %) using sheep anti-horse-HRPO. The Wb results were not always consistent with those of the ELISA analyses (e.g., some serum samples, which were negative in ELISA appeared positive in Wb and vice versa, Fig. 1). In Rome, only 16 (21.6 %) serum samples, which tested positive in Romania, were positive by both ELISA and Wb using the ES antigen; however, all these 16 samples showed optical density (OD) values similar to those of negative controls when they were examined in ELISA using the synthetic tyvelose glycan-BSA antigen. In contrast, positive control samples from six experimentally infected horses showed OD values 3-4 times higher than those of negative controls (data not shown).

DISCUSSION

The results suggest that ELISA and even Wb analyses utilizing an ES antigen could not well discriminate between specific and non-specific anti-Trichinella antibodies in horse sera (Fig. 1.). Consequently, the serological results obtained for horses from Romania could represent false positive reactions.

From the first finding of naturally infected horses in Mexico (Arriaga et al., 1995), important improvements were made in determining the optimal muscle and sample size for the direct parasite detection (Pozio et al., 1999; Boireau et al., 2000). On the other hand, the use of serology to detect Trichinella infection in horses remains a problem, mainly for two reasons: 1) the fast disappearance of circulating antibodies after infection...
The present results stress the need to standardize serological tests for each animal species, prior to use in epidemiological surveys. A similar problem occurred when 1 g of tissue from the diaphragm was used as a method to detect *Trichinella* infection in horses. This method, which was standardized to detect the infection in pigs, failed to detect the same parasite in horses (Pozio et al., 1999), in part, because the preferential muscles of this parasite in horses are the tongue and masseters (Gamble et al., 1996; Pozio et al., 1999).

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**REFERENCES**


