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 ELISA 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 ELISA 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 ELISA 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° 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 (Kirkegaard & 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

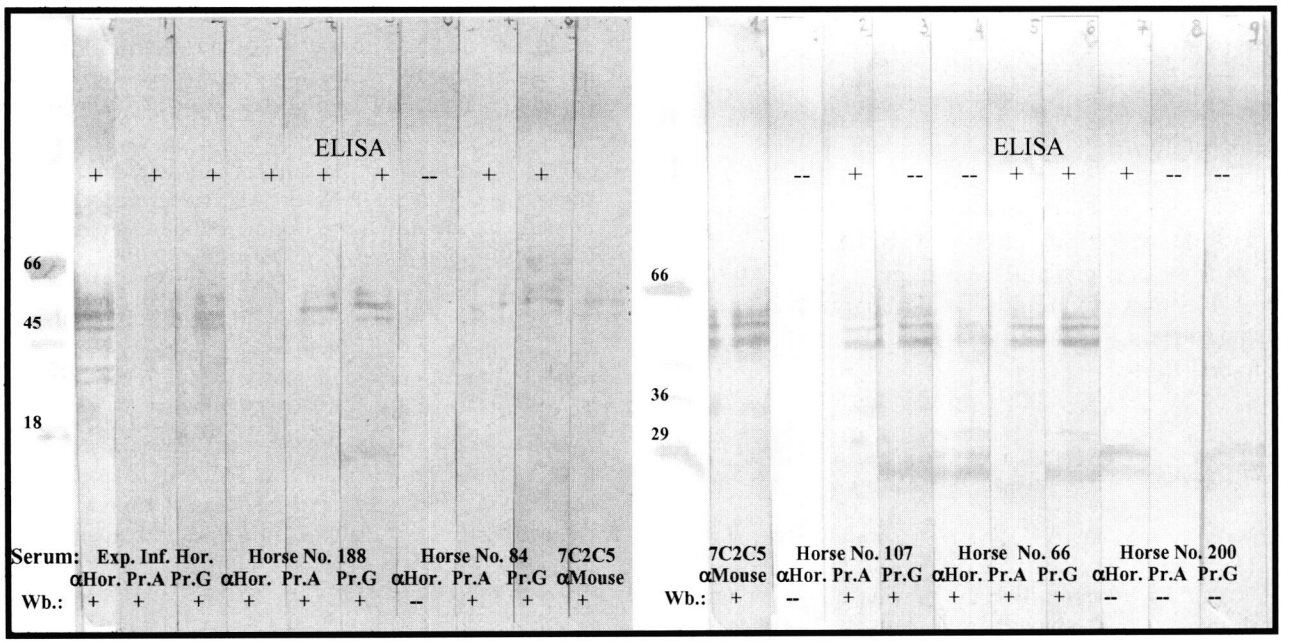

Fig. 1. – Western blot analyses of horse serum samples using the ES *Trichinella* antigen from *T. spiralis* L1 muscle larvae. Exp. Inf. Hor., serum sample from an experimentally infected horse showing a specific reaction of anti-*Trichinella* antibodies versus the ES antigen; Horse No.188, Horse No.84, Horse No.107, Horse No.66, Horse No.200, representative serum samples of horses from Romania showing unspecific reactions against the ES antigen; 7C2C5, monocolonal antibody (Gamble & Graham, 1984); conjugates: Hor, anti-Horse^{HRPO}; Pr.A, Protein A^{HRPO}; Pr.G, Protein G^{HRPO}.

(Soulé *et al.*, 1989; Pozio *et al.*, 1997; Boireau *et al.*, 2000); and 2) for the low specificity of ES antigen for horses, as reported here. In contrast, the ES antigen has proven to be highly specific for detection of circulating antibodies in humans (Murrell & Bruschi, 1994) and pigs (Gamble, 1998) infected with *Trichinella*.

Based on the data reported here, we suggest that previous findings of serological positive horses using ES antigen, in parasitologically negative horses (van Knapen & Franchimont, 1989; Arriaga *et al.*, 1997), could represent false positive test results.

From an epidemiological point of view, the lack of specific antibodies in 5,267 horses from Romania is consistent with the low incidence of *Trichinella* in horses during the 25 year-period from 1975 to 1999, recently estimated to be about 3.5/1 million horses slaughtered for human consumption (Pozio, 2000). In our opinion, the present results suggest that larger scale serosurveys (hundreds of thousands of horses) must be done in *Trichinella*-endemic regions to have the chance to detect positive horses.

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

ARRIAGA C., YEPEZ-MULIA L., VIVEROS N., ADAME L.A., LICHTENFELS J.R., ZARLENGA D., BENITEZ E. & ORTEGGA-PIERRES M.G. Detection of *Trichinella spiralis* in slaughter horses by artificial digestion and immunoenzymatic methods, *in*: Trichinellosis ICT9. Ortega-Pierres G., Gamble H.R., van Knapen F. & Wakelin D. (eds), Centro de Investigacion y Estudios Avanzados IPN, D.F. Mexico, Mexico, 1997, 441-445.

ARRIAGA C., YEPEZ-MULIA L., VIVEROS N., ADAME L.A., ZARLENGA D., LICHTENFELS J.R., BENITEZ E. & ORTEGGA-PIERRES M.G. Detection of *Trichinella spiralis* muscle larvae in naturally infected horses. *Journal of Parasitology*, 1995, *81*, 781-783.

BOIREAU P., VALLEE I., ROMAN T., PERRET C., FABIEN J.F., MINGYUAN L., GAMBLE H.R. & GAJADHAR A. Horse trichinellosis: a low frequency for a high human risk. *Veterinary Parasitology*, 2000, *93*,309-320.

GAMBLE H.R. Sensitivity of artificial digestion and enzyme immunoassay methods of inspection for trichinae in pigs. *Journal of Food Protection*, 1998, *61*, 339-343.

GAMBLE H.R., GAJADHAR A.A. & SOLOMON M.B. Methods for the detection of trichinellosis in horses. *Journal of Food Protection*, 1996, 59, 420-425.

GAMBLE H.R. & GRAHAM C.E. Monoclonal antibody-purified antigen for the immunodiagnosis of trichinosis. *American Journal of Veterinary Research*, 1984, *45*, 67-74.

GAMBLE H.R., RAPIC D., MARINCULIC A. & MURRELL K.D. Influence of cultivation conditions on specificity of excretory-secretory antigens for the immunodiagnosis of trichinellosis. *Veterinary Parasitology*, 1988, *30*, 131-137.

MURRELL K.D. & BRUSCHI F. Clinical trichinellosis, *in*: Progress in clinical parasitology. Sun T. (ed), CRC Press, Boca Raton, 1994, 117-150.

MURRELL K.D. & POZIO E. Trichinellosis: The zoonosis that won't go quietly. *International Journal for Parasitology*, 2000, *30*, 1339-1349.

OLTEANU GH. New Studies on epidemiology and control of trichinellosis in Romania. *In*: Trichinellosis ICT9. Ortega-Pierres G., Gamble H.R., van Knapen F. & Wakelin D. (eds), Centro de Investigacion y Estudios Avanzados IPN, D.F. Mexico, Mexico, 1997, 531-537.

POZIO E. Is horse-meat trichinellosis an emerging disease in EU? *Parasitology Today*, 2000, *16*, 266.

POZIO E., PATERLINI E., PEDARRA C., SACCHI L., BUGARINI R., GOFFREDO E. & BONI P. Predilection sites of *Trichinella spiralis* larvae in naturally infected horses. *Journal of Helminthology*, 1999, *73*, 233-237.

POZIO E., TAMBURRINI A., SACCHI L., GOMEZ MORALES M.A., CORONA S., GOFFREDO E. & LA ROSA G. Detection of *Trichinella spiralis* in a horse during routine examination in Italy. *International Journal for Parasitology*, 1997, *27*,1613-1621.

SOULÉ C., DUPOUY-CAMET J., GEORGES P., ANCELLE T., GILLET J.P., VAISSAIRE J., DELVIGNE A. & PLATEAU E. Experimental trichinellosis in horses: biological and parasitological evaluation. *Veterinary Parasitology*, 1989, *31*, 19-36.

VAN KNAPEN F. & FRANCHIMONT J.H. *Trichinella spiralis* infection in horses. *In*: Trichinellosis. Tanner E., Martinez Fernandez A.R. & Bolas Fernandez F. (eds), Consejo Superior de Investigaciones Cientificas Press, Madrid, Spain, 1989, 376-381.