DETECTION OF *TRICHINELLA* INFECTION IN SLAUGHTER HORSES BY ARTIFICIAL DIGESTION, ELISA AND PCR

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Summary :

In this study we compared the sensitivity of molecular, serologic and parasitologic methods for diagnosis of equine trichinellosis in two abattoirs, one rural and one federal inspection type. Diaphragm muscle samples were obtained from 170 slaughter horses and examined by artificial digestion and PCR. Serum samples from these horses were also analyzed by ELISA. No *Trichinella* muscle larvae were detected by artificial digestion. However, specific antibodies against *Trichinella* were detected in 17 % and 7 % of the serum samples examined from the rural and the federal abattoirs respectively. By PCR, 15 % and 2 % of the samples from these two abattoirs were *Trichinella* positive.

KEY WORDS : trichinellosis, horse, PCR.

Human trichinellosis outbreaks related to the consumption of horsemeat have been reported in France and Italy (Pozio *et al.*, 1997). However, the diagnosis of equine trichinellosis by direct methods has proven to be difficult, possibly due to low infections in which the amount of sample analyzed may influence the parasite recovery, or to different parasite tissue predilection sites which depend on the level of infection (Gamble *et al.*, 1996; Pozio *et al.*, 1999). On the other hand, previous studies of horses naturally infected with *Trichinella* have shown that serologic assays such as ELISA and Western blot do not allow detection of all infected horses (Pozio *et al.*, 1997; Yépez Mulia *et al.*, 1999). In some of these cases more sensitive and specific diagnosis could be achieved using molecular techniques. In particular, amplification of *Trichinella* DNA by the polymerase chain reaction (PCR) has been successfully used to detect the parasite in experimentally infected mice and horses (Dupouy-Camet *et al.*, 1991; Soulé *et al.*, 1994; Uparanukraw & Morakote, 1997).

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In order to compare the sensitivity of parasitologic, serologic and molecular methods in the diagnosis of equine trichinellosis, diaphragm tissue samples from 170 slaughter horses were examined by artificial digestion and PCR. In addition, serum samples from the same animals were analyzed by ELISA. The results suggest that the PCR technique could help to identify infected animals not detected by other diagnostic methods.

MATERIAL AND METHODS

TISSUE AND SERUM SAMPLES

Serum and diaphragm muscle tissue samples were collected from 170 horses slaughtered at two different abattoirs, one federal inspection type and one rural where naturally *Trichinella* infected horses had been previously detected. About 60 g of each sample were minced, 40 g for digestion and 1 g for PCR.

ARTIFICIAL DIGESTION (AD)

Tissue samples (40 g) were digested in 1 % pepsin-HCL for three hours at 37° C and the larvae recovered by sedimentation as described by Dennis *et al.* (1970). Muscle tissue samples from a horse experimentally infected with *T. spiralis* in which 16 larvae per gram (LPG) had been detected, were used as positive control for this procedure.

ELISA

Serum samples diluted 1:20 were analyzed by ELISA using excretory/secretory (E/S) products as described by Yépez-Mulia *et al.* (1999).

DNA EXTRACTION

One g of ground frozen tissue sample was incubated with 5 ml of lysis buffer (0.5M Tris, 0.2M EDTA, 0.5 % Sarkosyl and 10 µg/µl DNase free Rnase) for one hour at 37°C and with Proteinase K (100 µg/µl), for two

hours at 55°C and one hour at 65°C. One fifth (1 ml) of the supernatant was extracted with phenol-chloroform-isoamyl alcohol (25:24:1). The DNA was precipitated overnight with isopropanol and dissolved in 50 µl of water.

PCR

PCR was performed in 50 µl reaction volume containing 500 ng of template DNA, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 5 mM KCl, 0.1 % Triton X-100, 0.1 % gelatin, 200 µM each dNTP, 0.5 µg of each pPRA primers (Dupouy-Camet *et al.*, 1991) and 1.25 U of *Taq* polymerase (Amplitaq, Perkin-Elmer Corp, San Jose, Cal.). Amplification consisted of 30 cycles of one minute denaturation at 94°C, one minute annealing at 55°C and two minutes extension at 72°C, followed by seven minutes extension at 72°C. The amplification products were separated by electrophoresis on a 2 % agarose gel in Tris-acetate-EDTA buffer. Positive controls included DNA from *T. spiralis* muscle larvae (ML) and DNA from non infected horse muscle tissue spiked with 1,200 ML of *T. spiralis*. Negative controls were DNA from the non infected horse muscle tissue and a reagent control which included all PCR reagents except DNA.

RESULTS

Trichinella ML were not recovered by AD in any of the diaphragm muscle tissue samples from slaughter horses examined although ML could be detected in samples of the experimentally infected horse. However, when specific antibodies against *T. spiralis* in the corresponding serum samples were evaluated by ELISA using E/S antigens, 14/80 (17 %) samples from the rural abattoir and 6/90 (7 %) from the federal abattoir gave positive OD values.

The limit of sensitivity of PCR with pPRA primers was determined using double dilutions of 500 ng of DNA extracted from the non-infected horse tissue sample spiked with 1,200 ML. As little as 7 ng of *T. spiralis* DNA (corresponding to 0.06 ML) were detected. In addition, a sensitivity of 0.045 ML was determined when DNA extracted from tissue samples of a naturally infected horse harboring nine LPG was used. When muscle tissue samples from slaughter horses were analyzed by PCR, 2/90 (2 %) of the federal abattoir and 11/80 (14 %) of the rural slaughterhouse gave the expected amplification products of 600 and/or 800 bp (Fig. 1) as well as an additional band of 1,200 bp in some of these samples (Fig. 1, lanes 5-9). These bands were also observed with the DNA from isolated *T. spiralis* ML and with DNA from horse tissue samples spiked with ML used as positive control (Fig. 1, lanes 10-11). No amplification products were observed with DNA from tissue samples of 157/180 slaughter horses (representative data are shown in Fig. 1, lanes 12 and 13). The data obtained in AD, ELISA and PCR are summarized in Table I.

Type of abattoir	AD Positive/Total	ELISA Positive/Total (%)	PCR Positive/Total (%)
Rural	0/80	14/80 (17)	11/80 (15)
Federal	0/90	6/90 (7)	2/90 (2)

Table 1. – Results of artificial digestion (AD), ELISA and PCR in slaughter horses.

DISCUSSION

Detection of *Trichinella* ML in infected horses by parasitologic methods has been difficult, probably due in some cases to low parasite burden and to the localization of the parasite in defined muscle tissue (Gamble *et al.*, 1996; Pozio *et al.*, 1999). In fact, only in recent years detection of *T. spiralis* in naturally infected horses has been reported (Arriaga *et al.*, 1995; Pozio *et al.*, 1997) and in these studies large muscle tissue samples were used. Considering that serologic methods (ELISA and Western blot) are not useful to identify all infected horses (Pozio *et al.*, 1997, Yépez-Mulia *et al.*, 1999), we evaluated PCR for diagnosis of trichinellosis in muscle tissue samples from slaughter horses, compared with AD and ELISA. *Trichinella* larvae were not isolated in these animals by

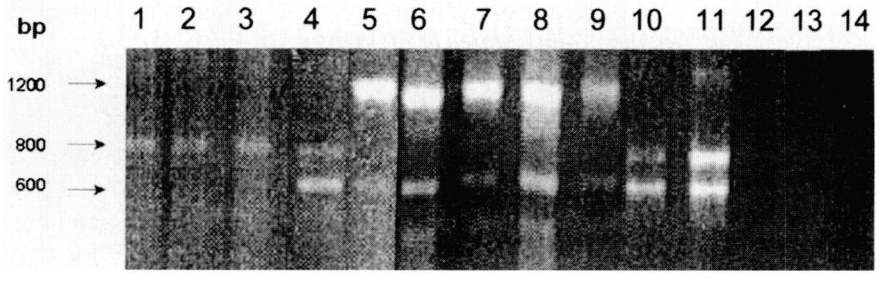

Fig. 1. – DNA samples amplified by PCR with pPRA primers. Lanes 1-4, positive samples from federal abattoir. Lanes 5-9, positive samples from rural slaughterhouse. Lane 10. Sample form non infected horse spiked with *T. spiralis* larvae. Lane 11. *T. spiralis* DNA. Lane 12, negative sample from federal abattoir. Lane 13, negative sample from rural abattoir. Lane 14, control reagents.

AD. However, by PCR some horse tissue samples gave the specific amplification products of 600 and 800 bp. In some cases, a band of 1,200 bp was observed, in agreement with the finding of Dick *et al.* (1992) using DNA samples from different *Trichinella* isolates. Since no amplification was observed in tissue samples of the non infected horse and in other samples of slaughter horses it seems that the animals positive by PCR were indeed infected. Considering that the PCR used in this study allowed the detection of 7 ng of DNA which correpond to 0.06 *T. spiralis* ML, it is possible that the parasite load in the diaphragm of these animals was below the limit of detection of AD since no *Trichinella* larvae could be found in these tissue samples. It is also possible that the partial destruction of the larvae by the horse immune system did not allow larvae recovery by AD although DNA could still be detected by PCR.

On the other hand, only three of the 20 animals positive by ELISA gave the expected amplification products by PCR. Some of the positive results obtained only by ELISA could be due to cross reactivity of TSL-1 antigens with other nematode antigens (De Ayuela *et al.*, 2000) as such antigens are highly represented in E/S products. In addition, other 11 samples negative in ELISA gave positive results by PCR, suggesting an early or late infections in those animals in which low antibody levels were possibly present as has been previously shown by Yépez-Mulia *et al.* (1999). These results provide further evidence that ELISA is not an adequate diagnostic test for equine trichinellosis.

Although it was not possible to recover the parasite in the diaphragm samples examined, it is possible that in other tissues the number of ML was higher, as mentioned by others (Gamble *et al.*, 1996; Pozio *et al.*, 1999). Head muscles are at the present recommended for the diagnosis of horse trichinellosis, however diaphragm tissue samples are still used for inspection in many abattoirs. Thus, it is important to have a very sensitive diagnostic method such as PCR that would allow the identification of infected horses not detected by other methods.

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