EARLY DETECTION OF *TRICHINELLA SPIRALIS* INFECTION BY THE POLYMERASE CHAIN REACTION IN BLOOD SAMPLES OF EXPERIMENTALLY INFECTED MICE

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Summary :

Up to now, there is no useful method to diagnose trichinellosis at the early stages of infection. The aim of the present investigation was to know if the polymerase chain reaction (PCR), could detect DNA of migratory larvae in mice experimentally infected with *T. spiralis*. Thirty-three Balb/c female mice 4.6 weeks old, were infected with 300 larvae of *T. spiralis* /mouse. Blood samples of these animals were positive for *T. spiralis* at 3th and 17th post-infection days. The control group was negative. PCR could detect from one to 200 larvae. Our results showed that it was possible to make early diagnosis of trichinellosis in blood of mice infected with *T. spiralis*.

KEY WORDS : *T. spiralis*, trichinellosis, PCR, DNA.

Trichinellosis is an endemic disease in some parts of the world caused by nematodes of the genus *Trichinella*, which are able to infect all kind of mammals, (1983). In Mexico, it has been reported as a sporadic disease by Martínez-Marañón *et al.* (1979), Fragoso (1981). Detection of specific DNA from *T. spiralis*, might be an accurate and sensitive method for detecting these parasite at early stages of the infection. Robert *et al.* (1996) assessed the validity of the PCR method in human and mice blood, Dupouy-Camet *et al.* (1991), used the PCR technique for the detection of a repetitive sequences of *T. spiralis* in experimentally infected mice. Dick *et al.* (1992), developed PCR primers based on sequences from a 1.6 kb DNA gene (gene pPRA described by de Vos *et al.*, 1988) to identify porcine isolates of *T. spiralis* from a single larvae from muscle tissue. Since then, the PCR has been proposed as an important and complementary technique for diagnosis of trichinellosis. The purpose of this study was to determine by PCR, the earliest time in which DNA from migratory larvae could be detected in blood of mice experimentally infected with *T. spiralis*.

MATERIAL AND METHODS

The *T. spiralis* isolate used in these experiments was from a naturally infected pig obtained from the Research Center and Advanced Studies (CINVESTAV, Mexico) and maintained by serial passages of 700 first-stage larvae (L1) in female Wistar rats. *T. spiralis* were isolated from infected rats, 30 days after infection by the standard pepsine hydrochloride digestion Blair (1983). Larvae were recovered by a modified Baerman apparatus, washed in PBS three hours at 37°C, and counted.

MICE INFECTION AND BLOOD COLLECTION

Thirty-three female Balb/c mice, 4.6 weeks old were infected orally with 300 larvae (L1); blood samples were collected at day 0. A similar number of mice were used as control group. Three animals from each group, were sampled and killed 3, 5, 7, 10, 12, 15, 17, 20, 22, 30 and 35 days post-infection. One ml of blood was collected in 0.5 M EDTA from the vena cava and processed for DNA extraction by phenol-chloroform isoamyl alcohol (Maniatis *et al.*, 1982).

POLYMERASE CHAIN REACTION

The PCR was performed in a 50 µl volume with 1.5 mM MgCl₂; 0.2 mM dNTPs; 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 500 ng of DNA and 1.25 unit of Taq DNA polymerase. The pPRA primers used were as follows: 5'-CTTGTAAGCGGTGGGCGTA^{3'} and 5'-CATAGAGGAGCAACATTACCT^{3'}, de Vos *et al.* (1988). The amplified products were run in 1.5 % agarose gels stained with ethidium bromide 0.05 % and then transferred on to Hybond-N nylon membrane (Amersham) and hybridized according to Maniatis *et al.* (1982), with a specific probe labeled with a fluorescein labeling kit (Life Science Products).

RESULTS

The PCR using the pPRA primers detected DNA of *T. spiralis* in blood samples of infected mice from day 5th until day 17th. All control groups

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were negative (Fig. 1A). Samples tested later, and until the 35th day post-infection, were negative (Fig. 2B). The nature of the amplified products was confirmed with a specific probe (Figs 1a-2b). The minimal amount of

DNA detected by the PCR ranged from 100 to 500 ng (Fig. 3A) and the probe could detect 90 pg of DNA of the parasite (Fig. 3a). It was possible to detect from one to 200 larvae by PCR (Fig. 4A).

Fig. 1A. *T. spiralis*: – PCR products were analysed by electrophoresis in 1.5 % agarose gel, days (0-12). M: Molecular weight. Line 1: day 15 line 2: day , line 4: day 5 line 6: day 7 line 8: day 10 and line 10: day 12. Negative controls: line 3: day 3, line 5: day 5, line 7: day 7, line 9: day 10 and line 11: day 12. Line 12: positive control and line 13: negative control.

Fig. 1a. – Southern blot hybridization with a fluorescein labeled probe of the same gel.

Fig 3A. – *T. spiralis*: PCR products were analysed by electrophoresis in 1.5 % agarose gel. M: Molecular weight. Line 1: 500 ng; line 2: 300 ng; line 3: 200 ng; line 4: 100 ng; line 5: 50 ng; line 6: 30 ng; line 7: 12 ng; line 8: 2 ng; line 9: 500 pg; line 10: 100 pg and line 11: 90 pg. Line 12: positive control and line 13: negative control.

Fig. 3a. – Southern blot hybridization with a fluorescein labeled probe of the same gel.

Fig. 2B. – *T. spiralis*: PCR products were analysed by electrophoresis in 1.5 % agarose gel, days (15- 35). M: Molecular weight. Line 1: day 15 line 3: day 17 line 5: day 20 line 7: day 22 line 9: day 30 and line 11: day 35. Negative controls: line 2: day 15 line 4: day 17 line 6: day 20 line 8: day 22 line 10: day 30 and line 12: day 35. Line 13: positive control and line 14: negative control.

Fig. 2b. – Southern blot hybridization with a fluorescein labeled probe of the same gel.

Fig. 4A. – *T. spiralis*: PCR products were analysed by electrophoresis in 1.5 % agarose gel. M: Molecular weight. Line 1: 200 larvae; line 2: 100 larvae; line 3: 80 larvae; line 4: 70 larvae; line 5: 60 larvae; line 6: 50 larvae; line 7: 20 larvae; line 8: 10 larvae; line 9: 5 larvae; line 10: 1 larvae. Line 11: Positive control and line 12: negative control.

Fig. 4a. – Southern blot hybridization with a fluorescein labeled probe of the same gel.

DISCUSSION

By using PCR technique, it was possible to detect *T. spiralis* larvae in experimental infected mice as early as the 5th day. This results is compatible with the life cycle of the parasite (Despommier *et al.*, 1995). The PCR results were positive from day 5 until day 17, but the hybridization was positive at day 3. These results were not very different from the ones reported by Soulé *et al.* (1993) in horses and Uparanukraw *et al.* (1997) in mice, since they reported amplification from day 5 to day 14 post-infection using pPRA primers. However, Robert *et al.* (1996) detected amplified products on the 7th day post-infection. The discrepancies between these authors are likely due to the DNA extraction procedure from blood samples and/ or to different parasitic burdens.

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