

DETECTION BY POLYMERASE CHAIN REACTION OF *TRICHINELLA SPIRALIS* LARVAE IN BLOOD OF INFECTED PATIENTS

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Summary :

Detection of the repetitive sequence pPra specific for *Trichinella spiralis* was carried out by PCR in the blood of 37 patients infected four to six weeks previously, during a horse-meat related outbreak of trichinellosis (Paris, 1993). Only two patients of 37 were PCR-positive. Human blood was sampled during the febrile phase of the disease, before any antihelminthic treatment. To assess the validity of the method, blood was taken from mice, 7, 10, and 13 days after infection by a *Trichinella spiralis* isolate obtained during the human outbreak. *Trichinella* DNA was detected in blood of mice, but only on day 7 post infection.

KEY WORDS : *Trichinella*, trichinellosis, PCR.

Résumé :

DÉTECTION PAR POLYMERASE CHAIN RÉACTION DE LARVES DE *TRICHINELLA SPIRALIS* DANS LE SANG DE PATIENTS INFECTÉS
Des PCR ont été réalisées dans le sang veineux de 37 patients infectés par *Trichinella spiralis* quatre à six semaines plus tôt au cours d'une épidémie provoquée par de la viande de cheval (Paris, 1993). La cible était la séquence répétée pPra, spécifique de *Trichinella spiralis*. Seuls deux patients ont eu une PCR positive. Les prélèvements avaient été effectués durant la phase fébrile et avant tout traitement anthelminthique. Pour vérifier la validité de notre méthode, du sang a été prélevé chez des souris infectées 7, 10 ou 13 jours auparavant par le même isolat de *Trichinella*. De l'ADN de *Trichinella* a pu être détecté dans le sang des souris mais seulement sept jours après l'infection.

MOTS CLÉS : *Trichinella*, trichinellrose, PCR.

INTRODUCTION

The polymerase chain reaction (PCR) has been used to detect *Trichinella* in muscle samples (Dupouy-Camet *et al.*, 1991) and to type isolates by means of either conventional PCR (Dick *et al.*, 1992; Soulé *et al.*, 1993a) or random amplification of polymorphic DNA fragments (RAPD), which can distinguish the different *Trichinella* species (Bandi *et al.*, 1993; Dupouy-Camet *et al.*, 1994a). Recently, Soulé *et al.* (1993b) used PCR to detect larvae in the blood of experimentally infected horses. We report here the use of PCR to detect larvae in blood of patients infected during the 1993 Paris outbreak related to horse-meat consumption (Dupouy-Camet *et al.*, 1994b). To assess the validity of our PCR method, experimental infections were also carried out in mice.

MATERIALS AND METHODS

PATIENTS

Venous blood (5 to 10 ml) was collected from 37 hospital patients during the febrile phase of trichinellosis (four to six weeks after the presumed date of infection), and before any specific treatment (thiabendazole), and from three controls free of trichinellosis. Specific antibodies were detected (indirect immunofluorescence on frozen sections of *Trichinella*) in 23 of the 35 patients tested. Twelve infected patients were seronegative, because tested too early. The three controls were also seronegative.

MICE

Mice (OF1 Swiss males, Iffa Credo, France) were infected by the isolate obtained from a muscle biopsy specimen of a cat infected during the outbreak and typed as *T. spiralis* by E. Pozio by means of PCR and RAPD techniques (*Trichinella* Reference Center, Istituto Superiore di Sanita, Rome). Mice were divided into four groups of six and were inoculated per os with 100 *T. spiralis* larvae, 200 larvae, 400 larvae, or no larvae (control group). Two animals of each group were killed 7, 10, and 13 days post-infection, and 1 ml of blood was taken from the vena cava. The small intestine was examined for adults by conventional methods (Blair, 1983).

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DNA PREPARATION

DNA was prepared from blood collected on EDTA (0,235 mol/l), with or without sedimentation on Histopaque (Sigma chemicals co, Le Perray-en-Yvelines, France). DNA of patients was prepared from the WBC (white blood cells) pellets obtained after sedimentation. DNA from blood mice was prepared from WBC pellets or from whole blood. DNA was obtained from whole blood, by classical phenol-chloroform extraction (Maniatis *et al.*, 1982); DNA was extracted from WBC pellets by using a simplified procedure including precipitation with 6M NaI and a purification with chloroform-isoamylalcohol (24/1 vol/vol) (Loparev *et al.*, 1991).

POLYMERASE CHAIN REACTION

The PCR target was the 1.6 kb repeat sequence pPra that is specific for *T. spiralis* (Klassen *et al.*, 1986; de Vos *et al.*, 1988), a sequence used in our laboratory to detect *Trichinella* in muscles samples. The sensitivity of this technique was previously evaluated in our laboratory : the 2800-fold repeated sequence could be detected in a DNA preparation corresponding to 1/50 larva (Dupouy-Camet *et al.*, 1991). PCR amplification was carried out with the same previously published oligonucleotides (5'GTAAAGCGGTGGTGCATTCAT and 5'-AGTTTCATACCGAACACCGCTC) and procedures, in a 100 ml reaction mixture containing 1 µg of DNA, 500 µM each dNTP and 2.5 U of *Taq* polymerase (Perkin-Elmer-Cetus, Saint-Quentin-en-Yvelines, France). The PCR products were separated through a 3 % agarose gel, stained with ethidium bromide and visualized by UV transillumination. The bands were compared with a molecular weight marker (pBR 328, *Bgl*I, *Hinf* I, Boehringer Mannheim, Meylan, France) and a positive control, consisting of genomic DNA from *T. spiralis* isolate TRLL 86 (ISS 104).

RESULTS

Only two patients of the 37 tested were PCR-positive. The three controls were negative. The PCR-positive patients did not differ from

the other patients with regards to treatment or clinical manifestations.

Trichinella spiralis DNA was detected in mouse blood on day 7 post-infection whatever the inoculum, but was no longer detected by day 10. Adult forms were only present in the small intestine on day 7. Results obtained with DNA from pelleted WBC were better than those obtained with whole blood (Table I).

DISCUSSION

The poor diagnostic performance of PCR on blood samples from patients infected by *Trichinella* is not surprising as blood was drawn during the muscular phase of the disease (four to six weeks after contamination), i.e., after the prepatent phase during which larvae are produced. In addition, the parasite burden during this outbreak was low, as only one and two larvae per 100 mg of muscle were observed in the two biopsy samples we were able to perform on two patients (not included in this study). However, the positivity of PCR in two patients is amazing because these patients apparently did not differ from the others. In these patients, the clearance of shedding females from the digestive tract could have been delayed for unknown reasons.

Technical problems due to DNA sample preparation as an explanation for the low sensitivity of PCR in patients blood, were ruled out by assessing our method in an experimental infection. Two types of DNA extraction were carried out during experimental infection; in this case DNA prepared from WBC pellets gave better results than DNA prepared from whole blood. The lower sensitivity of PCR on DNA from whole blood could be explained by the following hypothesis: *i*) presence of remaining inhibitors, such as heme, *ii*) DNA degradation due to the more aggressive extraction procedure. Moreover, the failure to detect *Trichinella* DNA in mice after the 7th day post-infection is in agreement with results of Bell and Wang (1987), who showed that more than 75 % of larvae

Inoculum	Day 7 PI *			Day 10 PI *		Day 13 PI *		
	PCR whole blood	WBC pellet	Adults in intestine	PCR whole blood	WBC pellet	PCR whole blood	WBC pellet	Adults in intestine
100 larvae	-	+	+	-	-	-	-	-
200 larvae	+	+	+	-	-	-	-	-
400 larvae	-	+	+	-	-	-	-	-
Controls	-	-	-	-	-	-	-	-

* PI : post-infection.

Table I. — PCR detection of *Trichinella* DNA in blood from experimentally infected mice (days 7, 10, and 13) and detection of adults in the small intestine by light microscopy (days 7, and 13).

were produced in rats during the 6th, 7th and 8th days post-infection. Our results are different from those obtained by Soulé *et al.* (1993b), who were able to detect circulating *Trichinella* DNA in horse blood for one month after infection, but in this case, horses were inoculated with 20,000 larvae and DNA was extracted from 30 ml of blood.

In conclusion, PCR appears to be of little diagnosis value during the muscular phase of the disease in humans. However, the finding of two positive patients indicates that the shedding of larvae could be longer in some patients and could lead to higher muscular larval burdens.

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