ELSEVIER Veterinary Parasitology 111 (2003) 59–63

veterinary parasitology

www.elsevier.com/locate/vetpar

Antibody levels by indirect ELISA test in *Trypanosoma evansi* infected horses following treatment with quinapyramine sulphate

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Received 10 November 2001; received in revised form 25 September 2002; accepted 6 October 2002

Abstract

An ELISA test was used to determine the persistence of antibody levels in horses following treatment for *Trypanosoma evansi*. In 17 horses with *T. evansi* from two farms treated and cured with quinapyramine sulphate, ELISA antibody levels fell progressively post-treatment, but remained with positive results for 22.6 months in one horse, 12.8 months in a second, 4.1 months in another four and 2.3 months in three, whilst the rest became negative at 2.3 months. In two horses that suffered a post-treatment infection relapse the decrease in ELISA levels was only temporary, and a new increase in antibody levels was proven. The follow-up of these antibody levels could prove useful in clinical cases and in epidemiological studies, as well as for assessing the efficacy of drug treatment.

Keywords: *Trypanosoma evansi*; ELISA; Persistence of antibody levels; Quinapyramine sulphate

Trypanosoma evansi in the subtropical area of Argentina produces, in horses, a disease characterized by anemia and loss of condition, known as "Mal de Caderas" (Monzon et al., 1990). The infection is transmitted mainly by insects of the genera Tabanus and mortality is very high if specific treatment is not administered.

Diagnosis of the disease depends either on demonstrating directly the presence of trypanosomes, or indirectly by detecting parasite antigens or DNA by PCR amplification, or by the detection of specific *T. evansi* antibodies in horses (Brun et al., 1998; Monzon, 1993;

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0304-4017/02/$ – see front matter
PII: S0304-4017(02)00331-X

Monzon et al., 1995). Detection of antibodies is used in many clinical or epidemiological situations and importantly, for certification purposes required for international horse-trading. An ELISA test (Monzon, 2000) was recently validated, with results expressed in terms of percent positivity (PP) relative to a standard reference serum. In this test, a PP of 50 is considered as the cut-off threshold for differentiation between a positive and a negative result; sensitivity of the test was 95%, with an interval of confidence (IC) between 91 and 99.7%, and 98% specificity with an IC of 95 to 100%.

The present communication reports the persistence of antibody levels detected by this ELISA test (Monzon, 2000) in horses with *T. evansi* and treated with quinapyramine sulphate (QS). Previous work showed that specific antibodies to this haemoflagelate might persist for several months after successful treatment with suramine (Monzon, 1993), a drug that is no longer available.

Blood samples with or without anticoagulants (Monzon et al., 1990) were collected from 20 horses in an outbreak of "Mal de Caderas" that occurred on one farm (FI) in the northeast Province of Formosa. Each sample was analyzed by the *T. evansi* ELISA test as previously described (Monzon, 2000), by the standard parasitological detection methods, (SPDM), namely the Woo and Rogers (1974) method and by blood inoculation in a pair of mice, where the two rodents were considered as a single unit (Monzon et al., 1990).

Based on these results we divided these horses into two groups: Group 1, with 8 non infected horses which tested negative both to ELISA and to SPDM, and Group 2, with 12 infected horses (prevalence of infection 60%). Ten of the horses in Group 2 tested positive to both parasite and ELISA analysis, and 2 resulted positive only in the ELISA test. Throughout the study both Groups 1 and 2 grazed together on the same pasture. Both groups were treated with QS at the dose of 3–4 mg/kg (Monzon and Mancebo, 1999) and blood samples were collected from the 20 animals at 2.3, 4.1, 10.5, 12.8 and 22.6 months post-treatment (PT) to evaluate parasitaemia and antibody titers in ELISA tests. To avoid the risk of re-infections during the trial no other horses were allowed on the premises.

At 22.6 months PT, all horses in Group 1 remained negative both to ELISA and to parasite detection, and 11 horses from Group 2 were declared cured based in their consistently negative parasitological testing. However, as shown in Fig. 1, low-titer ELISA positive results were still present at 22.6 months PT in one horse (9%), at 4.1 months in other three (27%), and at 2.3 months in one horse (9%). The remaining six horses (54%), of Group 2, had become ELISA negative at 2.3 months. Coincidentally with an increase of antibody levels and decreased packed cell volume (PCV), one horse of Group 2 (horse N° 1) became parasitaemic again at 4.1 months PT, thus requiring additional QS treatment (Fig. 2). Unfortunately, this horse disappeared from the farm, and further studies were not possible.

We also studied 19 horses from a herd in a second farm (F2) located near to farm F1. All of these 19 field horses proved 100% positive to ELISA, with *T. evansi* isolated from 16 of them. All these 19 horses were treated with QS, as described with the F1 herd. Post-treatment blood samples were collected from 7 horses at 2.3, 4.1 and 12.8 months, with negative SPDM results in six of them and considered cured. In the last control, at 12.8 months, one horse (N° 2) showed clinical signs of the disease, and mice inoculated with its blood, developed parasitaemia, thus indicating infection (Fig. 2). However, in the other six cured horses, positive ELISA results persisted: two horses remained positive after 2.3

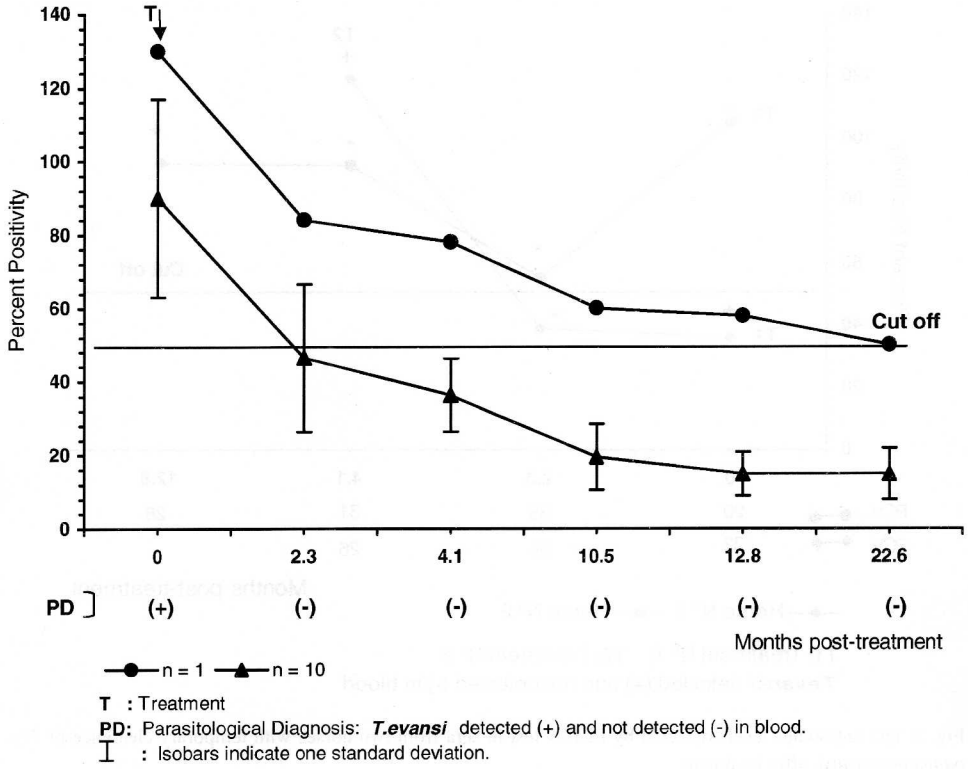

Fig. 1. IgG antibodies levels by ELISA test, in sera from field horses infected with *Trypanosoma evansi* and cured with quinapiramine sulphate.

months PT, one horse for 4.1 months, and 12.8 months in a fourth horse. A 4 month PT SPDM control performed in the remaining 12 horses from farm F2 gave negative results. However, measures to prevent reinfections were not possible in this farm.

Following treatment of *T. evansi* infected horses with suramine (Naganol), antibodies were detected in 3/10 horses six months PT (Monzon, 1993). Luckins et al. (1978) reported that the Micro ELISA values had declined by approximately 50% at 30 days PT in infected *T. evansi* rabbits, and were still detectable for several months. The present communication reports that horses with successful trypanocidal therapy show a progressive decrease of their antibody levels. In contrast, an increase in antibody titers was observed when the cure is only temporary, in agreement with ELISA results obtained in sera from a horse with experimental *T. evansi* infection in which a first treatment did not interrupt the evolution of the disease (Monzon, 2000). After trypanocide drug treatment, relapse may be attributed to the survival of drug resistant trypanosomes, or be the result of the parasite escaping drug action, possibly when they remain occult in the spinal fluid and later reinvade the circulation (Biswas and Hunter, 1993; Monzon et al., 1995), re-infections events occur generally when horses are challenged with an heterologous parasite population (Luckins, 1994; Nantulya et al., 1984).

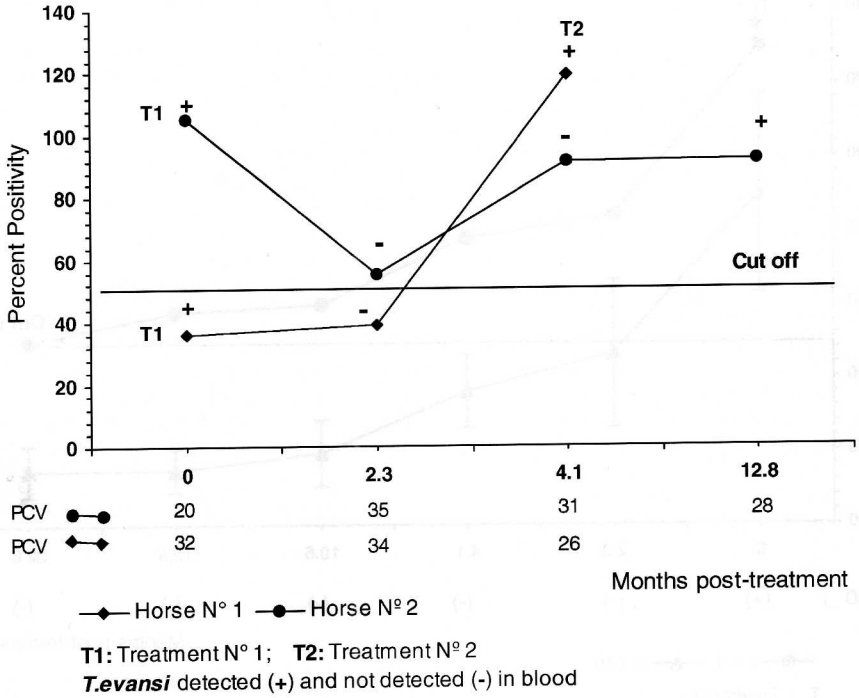

Fig. 2. IgG antibodies levels detected by ELISA test in sera from two horses with temporary clearence of *Trypanosoma evansi*, after treatment.

Titers of IgG antibodies are not able to differentiate between current and previous *T. evansi* infection, and for this reason it is proposed that a post-treatment follow-up by the ELISA test may constitute an auxiliary tool to evaluate the effectiveness of anti-trypanocidal drugs, especially if employed together with the detection of circulating antigens and SPDM. More so considering that the low sensitivity of these methods results in serious limitations (Monzon et al., 1990; Nantulya, 1990; Monzon et al., 1995). It is also concluded that relapses could occur after several months PT, thus suggesting that a long period of time is required in order to declare as trypanosome-free horses that are treated with anti-trypanosomal drugs.

Acknowledgements

The authors acknowledges the valuable help of Dr. G.Mauricio Bulman, Consultant in Veterinary Parasitology and Dr. Carlos Suarez, USDA-Animal Research Unit, for advises and corrections to the manuscript.

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