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Short communication

Repeated high dose imidocarb dipropionate treatment did not eliminate *Babesia caballi* from naturally infected horses as determined by PCR-reverse line blot hybridization

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Abstract

Imidocarb treatment of horses infected with *Babesia caballi* is supposed to eliminate the infection, but data on the efficacy of this treatment is scarce. The study presented here concerns four Paso Fino horses, which were imported into the island of Curacao on the basis of a piroplasmosis negative complement fixation test (CFT). Upon re-testing with an indirect fluorescent antibody test immediately after arrival in Curacao, two horses appeared to have antibodies to *B. caballi* and all horses had antibodies to *Theileria equi*. Subsequent testing with polymerase chain reaction combined with a reverse line blot yielded positive results for both agents in all four horses. Treatment with five consecutive doses of imidocarb dipropionate (4.7 mg/kg BW im q 72 h), temporarily resulted in negative results, but *B. caballi* and *T. equi* were detected again in the samples taken at 6 and 18 weeks after completion of the treatment. These results confirm that the CFT is not a suitable test for pre-import testing and that even high dose treatment with imidocarb may not be capable of eliminating *B. caballi* and *T. equi* infections from healthy carriers.

Keywords: Imidocarb treatment; Piroplasmosis; Horse; PCR-RLB

1. Introduction

Equine piroplasmosis, caused by *Babesia caballi* and *Theileria equi*, is an important tick-borne protozoan disease (Friedhoff et al., 1990). The agents are endemic throughout the (sub)tropics and stringent regulatory import restrictions are in place in some countries to prevent their spread among both resident and transient equid populations (Friedhoff et al., 1990). Equine piroplasmosis occurs in acute, sub-acute and chronic forms. Carriers of the infection are mostly asymptomatic. Identification of parasites in blood smears is the diagnostic mainstay, but this bears certain limitations, particularly when parasitaemia is low (Krause et al., 1996). Serodiagnosis by the use of complement fixation test (CFT) alone may give false negative test results, especially in horses that are parasite carriers, and has been shown to be less sensitive than the indirect

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Table 1
Results of PCR-RLB for *Babesia caballi* and *Theileria equi* in the blood of four horses during and after five consecutive Imidocarb treatments (4.7 mg/kg BW im q 72 h) commencing on 4 September 2002

Sample date	Horse 1		Horse 2		Horse 3		Horse 4	
	B. caballi	*T. equi*	*B. caballi*	*T. equi*	*B. caballi*	*T. equi*	*B. caballi*	*T. equi*
4 September 2002	+	+	+	+	+	+	+	+
7 September 2002	+	+	+	+	+	+	+	+
10 September 2002	−	+	−	+	−	+	−	+
13 September 2002	−	+	−	−	−	−	−	+
16 September 2002	−	+	−	−	−	−	−	+
30 October 2002	+	+	+	+	−	+	+	+
31 January 2003	+	+	+	+	+	+	+	+

+: positive result; −: negative result.

fluorescent antibody test (IFAT) (Donnelly et al., 1980; Frerichs et al., 1969; Tenter and Friedhoff, 1986; Weiland, 1986). Polymerase chain reaction (PCR) proved very useful for the detection of haemoparasites (Caccio et al., 2000), and combined with reverse line blot (RLB) offers the possibility of simultaneous detection and identification of different species infecting horses (Nagore et al., 2004). Imidocarb treatment of *B. caballi* infected horses has been shown to eliminate the infection (Bruning, 1996; Frerichs and Holbrook, 1974), but data on the efficacy of this treatment is scarce and more sensitive diagnostic tools have developed over the years. In an attempt to eliminate the infection from the horses in this study, treatment with a high dose of imidocarb dipropionate (4.7 mg/kg BW im q 72 h 5 times) was initiated and blood was tested using PCR-RLB to monitor the effect of treatment.

2. Materials and methods

A group of four Paso Fino horses, naturally infected with *B. caballi* and *T. equi*, ranging in age from 6 to 8 years, with a weight between 325 and 350 kg were treated five consecutive times with a high dose of imidocarb dipropionate (Imizol®[1] 4.7 mg/kg BW im q 72 h 5 times). Following treatment, blood samples were collected sequentially. The blood was stored at −20 °C, until DNA was extracted using a DNA extraction kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. The procedures for obtaining PCR products used for the detection of *Theileria* and *Babesia* species by RLB and the RLB hybridization itself have previously been described (Nijhof et al., 2005). Negative and positive control samples were included in every run. A standard IFAT protocol was used as previously described (Madden and Holbrook, 1968), using locally produced antigens. Bound equine antibodies were detected with fluorescein isothiocyanate-conjugated rabbit anti-horse immunoglobulin (RAHo/IgG(H + L)FITC, Nordic Immunology, Tilburg, the Netherlands) and examined in a wet mount by fluorescence microscopy.

3. Results and discussion

The study presented here concerns four Paso Fino horses, which were imported into the island of Curacao on the basis of a negative piroplasmosis CFT. Upon re-testing with IFAT immediately after arrival in Curacao, two horses appeared to have antibodies to *B. caballi* and all horses had antibodies to *T. equi*. Subsequent testing with PCR-RLB yielded positive results for both agents in all four horses. Travel-related stress might have affected them immunologically (Hailat et al., 1997), causing a flare up of the infection resulting in the positive PCR-RLB after arrival in Curacao. None of the horses showed clinical signs of disease during their stay in regulatory quarantine. Treatment with a high dose of imidocarb dipropionate (4.7 mg/kg BW im q 72 h 5 times) was initiated, and blood samples (EDTA, Vacuette®) were collected sequentially between 4 September 2002 and 31 January 2003. PCR-RLB results are given in Table 1. It is clear that *B. caballi*, and to a lesser degree *T. equi*, detection fails after two doses of the five-dose course but that they are detected again in the samples taken at 6 and 18 weeks after completion of the treatment. The lack of efficacy was surprising since a much lower dose of imidocarb 2 mg/kg q24 h twice) was previously reported to be capable of eliminating *B. caballi* (Frerichs and Holbrook, 1974). Detectable DNA actually disappeared from the circulation during the treatment and re-appeared later. Moreover, since dead agents/organisms are rapidly degraded and removed by warm blooded hosts and since

[1] Imizol®, Schering-Plough S.A., Friesoythe, Germany.

long time survival of DNA in the circulation seems incompatible with life, it is highly unlikely that the DNA of dead *B. caballi* organisms was detected at 6 and 18 weeks post-treatment. In addition, re infection is also an unlikely explanation for our findings since the tick species required for transmission did and do not occur in Curacao.

In conclusion, the results reported here confirm that the CFT is not a suitable test for pre-import testing and suggest that even high dose treatment with imidocarb may not be capable of eliminating *B. caballi* and *T. equi* infections from healthy carriers.

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