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Short communication

First report of *Trypanosoma vivax* infection in dairy cattle from Costa Rica

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ABSTRACT

An outbreak of haemoparasitoses occurred from October 2007 to July 2008 in cattle from the district of Rio Cuarto, province of Alajuela, Costa Rica. Fifty animals of various ages out of 450 Brown Swiss were affected. The animals presented fever, severe anemia, jaundice, abortion or premature birth, loss of appetite, decrease milk production and accentuated weight loss in a short period of time. Haemoparasites were observed in the blood smears: *Anaplasma marginale* was present in 17 animals (60.7%); *Trypanosoma vivax* in nine (32.1%) and *Babesia bovis* in two (7.1%). Three of the animals (10.7%) had a mixed infection with *T. vivax* and *A. marginale*. After treatment, all the animals were clinically recovered and subsequent blood samplings showed no parasites. Data suggest that the outbreak might be related to a decrease in the availability and quality of the pastures due to very heavy rainfalls during the year 2007, as well as an increase in the abundance of *Boophilus microplus* and *Stomoxys calcitrans*. This is the first report of the presence of *T. vivax* in Costa Rica.

1. Introduction

The protozoan *Trypanosoma* (Duttonella) *vivax* Ziemann, 1905, infects a wide range of wild and domestic ungulates. In Africa, it is transmitted cyclically by *Glossina* spp. and mechanically by other bloodsucking insects (Hoare, 1972; Gardiner, 1989; Silva et al., 1996; Jones and Dávila, 2001). *T. vivax* was introduced into the New World probably around 1830, when Zebu cattle from Senegal was imported to the French Guiana and Antilles and the parasite adapted to the mechanical transmission by tabanids (horse flies) and *Stomoxys calcitrans* (stable flies) (Shaw and Lainson, 1972; Clarkson, 1976; Gardiner, 1989; Paiva et al., 2000; Osório et al., 2008). Since its introduction, the natural infection with *T. vivax* has been reported in cattle from several countries: Panama, Surinam, Venezuela, Colombia, Brazil, Bolivia, Paraguay, Peru, and the Caribbean islands of Guadeloupe and Martinique (Johnson, 1941; Shaw and Lainson, 1972; Clarkson, 1976; Silva et al., 1998b; Quispe et al., 2003; Osório et al., 2008). In addition to the above-mentioned countries, serological evidence of infection with *T. vivax* has been reported in cattle of Costa Rica (22.9%) and El Salvador (15%) by Wells et al. (1977).

Infected cattle might be completely asymptomatic or can develop acute illness with some degree of mortality (Gardiner et al., 1989; Quispe et al., 2003; Batista et al., 2007). Reports of *T. vivax* outbreaks in South America informed that the main clinical signs are intermittent fever, anemia, abortion, apathy, loss of body condition and of appetite, decrease of milk production, progressive weight loss and death (Silva et al., 1996, 1998a; Silva and Dávila, 2001; Batista et al., 2007; Osório et al., 2008). Although outbreaks in cattle with these characteristics may be sporadic in endemic areas of Latin America, the trypanosomiasis should be taken into account as an

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economically important parasitic disease together with other haemoparasitoses such as babesiosis and anaplasmosis (Clarkson, 1976; Otte et al., 1994; Paiva et al., 2000; Quispe et al., 2003). Parasitism caused by *T. vivax* in Costa Rican cattle is reported for the first time in the country as a result of a seroepidemiological survey on anaplasmosis and babesiosis in dairy herds.

2. Materials and methods

The reported trypanosomiasis outbreak occurred in a dual purpose farm with approximately 900 animals: Brown Swiss, Simmental and crossbreeds. The farm has an area of 300 ha and is located at an altitude of 350 masl, district of Rio Cuarto de Grecia (10.34° latitude, 84.22° longitude), province of Alajuela. The zone is characterized by a tropical climate with a mean temperature of 23 °C (ranging from 15.8 to 31.1 °C) and a relative humidity between 80% and 90%. The annual precipitation is 2000 mm. Economically, the region is dedicated to livestock and agricultural activities such as pineapple and ornamental plants cultivation for exportation.

Whole blood samples were collected from the tail vein using a vacuum system and were kept on ice while transported to the laboratory. Blood smears from each sample were stained with Giemsa and trypanosomes identified based on morphological and biometrical data (Hoare, 1972).

The farm owner as well as a veterinarian was interviewed in order to obtained information about past and current health problems of the herd.

3. Results and discussion

From October 2007 to July 2008, 50 out of 450 Brown Swiss animals (ages ranging between 1 week and 15 years) presented fever, severe anemia, jaundice, abortion or premature birth, loss of appetite, decrease of milk production and accentuated weight loss in a short period of time. Haemoparasites were observed in 28 (56.0%) of the 50 sick animals: *Anaplasma marginale* was identified in 17 animals (60.7%), *T. vivax* in nine (32.1%) and *Babesia bovis* in two (7.1%). In addition, three (10.7%) of these animals had a mixed infection with *T. vivax* and *A. marginale*. In the present study, the diagnosis of haemoparasites was based on whole blood smears due to the fact that clinical cases were thought to be caused by *A. marginale* and *Babesia* spp., although microhematocrit is the most recommended technique for the diagnosis of acute infection caused by *T. vivax* (Woo, 1970). The reported blood smears sensitivity for the diagnosis of infections caused by *T. vivax* is 10% (Quispe et al., 2003), while Woo's technique has a sensitivity of over 60% (Desquesnes, 1997).

Trypanosomes found in this survey were morphologically pleomorphic (Fig. 1) showing a large rounded nucleus and a rounded posterior extremity; kinetoplast was subterminal and laterally positioned. Undulating membrane was poorly developed and the flagellum was short. Biometric analysis of parasites yielded the following dimensions: total length 18.80 ± 2.72 μm; distance from kinetoplast to posterior end 1.21 ± 0.56 μm; kinetoplast to nucleus length 5.2 ± 1.03 μm; posterior end to nucleus distance 6.5 ± 1.19; nucleus to anterior end distance 7.3 ± 1.13 μm and free flagellum length 4.5 ± 0.57 μm. These data show similarities with the report by Hoare (1972) and other authors in Latin America (Table 1).

Prevalence of *T. vivax* infection in cattle of the New World is difficult to determine because farmers and veterinarians generally treat the animals once clinical symptoms appear without the laboratory confirmation (Clarkson, 1976; Quispe et al., 2003). Upon diagnosis, the sick animals were treated with diminazene aceturate and/or oxytetracycline. Treated animals were clinically recovered and no haemoparasites could be observed in subsequent blood smears examinations.

In 1979, clinicians from the Large Animals Clinic (School of Veterinary Medicine, Universidad Nacional), attended several clinical bovine cases from the districts of Abangares, Bagaces and Nicoya, province of Guanacaste. These animals were prostrated and the farmers refer it as downer cow syndrome or "bovino caído" o "derrengue". The animals presented fever, weakness especially of the hind limbs, dehydration, sialorrhea and nervous signs such as hyperestesia, opisthotonos, and death occurred in some cases (Podestá-Morel, 1982; Evans-Benavides, 1983). Among differential diagnosis, bovine paralytic rabies, listeriosis, intoxication by *Melochia pyramidata* ("escobilla

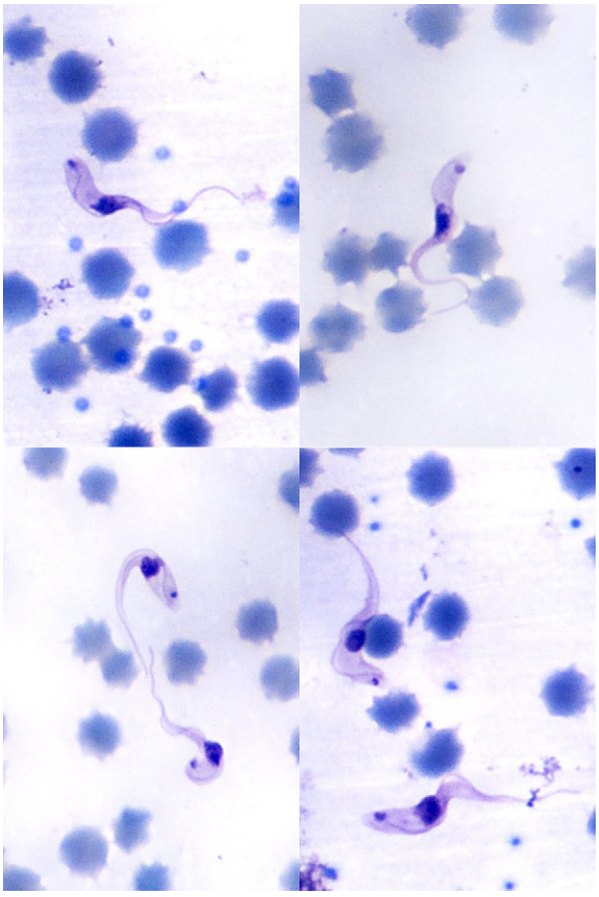

Fig. 1. *Trypanosoma vivax* from cattle of Costa Rica (100×).

Table 1
Comparative biometrical data (μm) of Costa Rican and some isolates of *Trypanosoma vivax*.

Isolates	*L*	PK	KN	PN	NA	*F*
Hoare (1972)	18–31	–	–	–	–	3–6
Panama (Johnson, 1941)	21.38	–	–	–	–	–
Brazil (Shaw and Lainson, 1972)	22.77 (1.38)	0.65 (0.25)	6.16 (0.57)	7.60 (0.57)	8.22 (1.08)	6.92 (1.03)
Brazil (Silva et al., 1996)	18.73 (3.8)	1.02 (1.16)	6.10 (1.29)	7.18 (1.18)	5.40 (1.63)	6.15 (2.38)
Bolivia (Silva et al., 1998b)	15.86 (2.23)	0.54 (0.51)	5.05 (1.07)	5.59 (1.15)	5.90 (0.76)	4.35 (1.26)
Bolivia (Silva and Dávila, 2001)	17.37 (1.65)	0.99 (0.51)	5.25 (0.68)	6.24 (0.83)	5.77 (0.68)	5.33 (0.83)
Guyana (Desquesnes, 2004)	20.3	–	–	–	–	–
Venezuela (Desquesnes, 2004)	21.5	–	–	–	–	–
Costa Rica (present study)	18.80 (2.72)	1.21 (0.56)	5.2 (1.03)	6.5 (1.19)	7.3 (1.13)	4.5 (0.57)

L, total length; PK, distance from kinetoplast to posterior end; KN, kinetoplast to nucleus length; PN, posterior end to nucleus distance; NA, nucleus to anterior end distance; *F*, free flagellum length.

morada") and trypanosomiasis by *T. vivax* were taken into account. Laboratory analysis yielded negative results for the two first diseases and only *T. theileri* was identified on blood smears. Despite the presence of *M. pyramidata* in all the farms with sick animals, plant intoxication could not be ruled out because autopsy of dead animals was not done. Evans-Benavides (1983) identified *T. theileri* in 46.5% (93/200) of blood samples from healthy cattle from slaughterhouses in Costa Rica. Based on this finding, the author concluded that *T. theileri* was probably the only species of *Trypanosoma* present on cattle, but the possibility of introducing of *T. vivax* into the Costa Rican territory was not ruled out. Later in 2003 *T. vivax* was identified in white tail deers (*Odocoileus virginianus*) from the province of Puntarenas by of us in blood smears brought by doctor Rita Coghi (unpublished data).

The clinical signs reported in this study have also been described in outbreaks of trypanosomiasis in the region of Pantanal (Brazil, Bolivia and Paraguay) (Silva et al., 1996, 1998a, 1998b; Silva and Dávila, 2001) French Guiana (Desquesnes and Gardiner, 1993); Colombia (Otte et al., 1994) and Venezuela (Clarkson et al., 1971; Tamasaukas and Roa, 1991–1992). In contrast to Costa Rica, mortality was observed in some of the reported outbreaks (Silva and Dávila, 2001; Osório et al., 2008). On the other hand, Paiva et al. (2000) did not detect clinical signs in animals infected with *T. vivax* in Pantanal the state of Mato Grosso do Sul, Brazil. In an outbreak reported in the northeast region of Brazil, 64 cows were affected and 11 of them died. Infected animals presented fever, anemia, weight loss and neurological alterations; rates of morbidity (49.2% adults and 32% young) and mortality (17.2% adults and 15% young) occurred due to the introduction of *T. vivax* into a susceptible Brown Swiss herd after an apparent increase of tabanid populations (Batista et al., 2007). In the present study none of the calves from the positive cows showed the parasite. Young animals (up to 12 months old) are more resistant to the infections caused by trypanosomes than adults being the former able to recover spontaneously (Uzoigwe, 1986). This may explain why only adult animals were found infected with *T. vivax* in the present study.

In the interview, both the owner and the veterinary indicated that the main sanitary problems in the farm were anaplasmosis, babesiosis, foot problems and leptospirosis which explain the previous use of oxytetracycline and diminazene aceturate for the control of *A. marginale* and *Babesia* spp. They mentioned also that the main ectoparasites found in the farm were *S. calcitrans* (stable flies) and ticks. Asked about the presence of tabanids, they were emphatic upon affirming that these insects are not frequent in the farm. Several authors indicate the association of the rainy season, when bloodsucking insects (particularly tabanids) are more abundant, with an increase in the prevalence of *T. vivax* in South America (Gardiner, 1989; Tamasaukas and Roa, 1991–1992; Silva et al., 1996, 1998b; Silva and Dávila, 2001; Batista et al., 2007). In addition to tabanids, Batista et al. (2007) called the attention upon the possible role of *Haematobia irritans* (horn flies) in an outbreak reported in Brazil. For the present study, it is important to emphasize the role of *S. calcitrans* as a probable mechanical vector of *T. vivax*, because in this and other districts of the country dedicated to pineapple cultivation *S. calcitrans* populations are constant along the year, with periods of higher abundance associated to the inadequate management of pineapple wastes. There are frequent reports of *S. calcitrans* causing many problems both to livestock and people living in near areas to this crop (SENASA, unpublished data).

The lesions as well as the clinical signs caused by *T. vivax* are common to several etiologies, such as those of nutritional, toxic and infectious origin; frequently *T. vivax* occurs together with other infections, complicating the diagnosis and causing higher morbidity and mortality (Paiva et al., 2000; Quispe et al., 2003; Osório et al., 2008). In the state of Mato Grosso do Sul, Pantanal of Brazil, *T. vivax* was considered as a secondary agent because the direct cause of morbidity and mortality was attributed to primary agents such as *A. marginale*, *Babesia* spp., plant intoxication and deficient management conditions (Paiva et al., 2000). Nevertheless, these authors suggest that under these circumstances *T. vivax* should not be excluded. The outbreak of haemoparasitoses (anaplasmosis, trypanosomiasis and babesiosis) reported in the present study, was probably a result of lower availability and quality of the pastures, an increase in the numbers and severity of lameness, and higher abundance of *Boophilus microplus* and *S. calcitrans*, associated to the rainy season of 2007. Probably, all the above-mentioned factors together with an increase in the rate of haemoparasites transmission were the triggering factors of the outbreak. The situation in the farm began to return to the normality after the end of the rainy season (December 2007) and once the animals

received the adequate treatment. Animals with good sanitary and nutritional conditions are able to maintain a balance with *T. vivax* infection (Paiva et al., 2000). It is noteworthy that in our case, there was another outbreak during the months of May–July 2008 (start of rainy season), which was associated to a large increase in the population of *S. calcitrans* as a result of the renewal of pineapple cultivations.

The serological evidence of infection with *T. vivax* has been reported in Costa Rica by indirect fluorescent antibody test (Wells et al., 1977), however this is the first report of the infection by *T. vivax* in cattle from Costa Rica. More studies are necessary to determine the epidemiology and the economic impact of the haemoparasitoses in cattle in Costa Rica especially those infections caused by *T. vivax*.

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