Molecular diagnosis of cattle trypanosomes in Venezuela: evidences of *Trypanosoma evansi* and *Trypanosoma vivax* infections

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Abstract In South America *Trypanosoma evansi* has been determined by molecular methods in cattle from Bolivia, Brazil, Colombia and Peru, reason for which the presence of this parasite is not excluded in Venezuelan livestock. Therefore, the aim of this study was to perform parasitological and molecular diagnosis of cattle trypanosomosis in small livestock units from two regions in this country. The parasitological diagnosis was carried out by MHCT and the molecular by PCR using genus-specific ITS1 primers that differentiate *T. vivax* and *T. evansi* infections. 47 cattle were evaluated in the “Laguneta de la Montaña” sector, Miranda State, where 3 animals were diagnosed as positive (6.4 %) by MHCT and 14 (30 %) by PCR as *Trypanosoma* spp., out of which 9 animals resulted positive for *T. vivax*, 3 for *T. evansi* and 2 with double infections. Whilst in the “San Casimiro” sector, State of Aragua, out of the 38 cattle evaluated 7 animals were diagnosed as positive (18.4 %) by MHCT and 19 (50 %) by PCR, determining only the presence of *T. evansi* in this locality. The molecular diagnosis by PCR using ITS1 primers allowed *T. evansi* detection in cattle field populations, which suggests the possible role of these animals as reservoirs in the epidemiology of the disease caused by *T. evansi* in Venezuela.

Keywords Molecular diagnosis · PCR · *Trypanosoma evansi* · *Trypanosoma vivax* · bovine trypanosomosis

Abbreviations

ITS1 Internal transcribed spacer 1
MHCT Micro-haematocrit centrifugation technique
PCR Polymerase chain reaction

Introduction

In South America the main species that causes animal trypanosomosis are *Trypanosoma vivax* and *Trypanosoma evansi*. *T. vivax* affects cattle, sheep, goats, and buffaloes, while *T. evansi* is particularly pathogenic in horses (Desquesnes 2004). Animal trypanosomosis represents a potential risk to nearly 350 million cattle (FAOSTAT database), 1.8 million buffaloes and 16 million horses in this region (Dávila and Silva 2000). Desquesnes (2004) estimated that an outbreak of trypanosomosis in beef cattle cost the equivalent of 3.3 % live weight per animal; which can be split into 30€ of weight loss and 3€ of treatment per head. These haemoparasites cause reproductive disorders such as infertility and abortions, as well as growth delay, weight loss and diminished physical condition that can lead to animal death (Desquesnes 2004; Gonzatti et al. 2014). In horses, *T. evansi* produces central nervous system disorders and muscle atrophy in the limbs which causes animal prostration (Desquesnes et al. 2013a) and death. However in South America, in domestic animals such as cattle, buffaloes, sheep and goats, *T. evansi* is considered as a low pathogenic agent, since those animals are asymptomatic or

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show partial clinical signs. This condition allows the classification of cattle and other animals as partial hosts or low susceptibility reservoirs (Desquesnes et al. 2013a, b). On the other hand, *T. evansi* infection in cattle has been reported in South American countries such as Bolivia, Brazil, Colombia and Peru (Franke et al. 1994; Dávila et al. 2003; Gonzales et al. 2003; Herrera et al. 2004; Cassalett et al. 2006; Gonzales et al. 2007; Mekata et al. 2009), reason for which the presence of this parasite is not excluded in Venezuelan cattle.

Besides the domestic hosts, *T. evansi* is found in a wide range of wild hosts (Desquesnes et al. 2013a). In South America, coatis (*Nasua nasua*), capybaras (*Hydrochoerus hydrochaeris*) (Franke et al. 1994; Arias et al. 1997) and vampire bats (*Desmodus rotundus*) (Dávila and Silva 2000) play an important role as reservoirs.

In Venezuela, animal trypanosomosis occurs endemically with high seroprevalence and wide distribution (García et al. 2000; Gonzatti et al. 2014). Generally, infected animals do not show clinical signs during the chronic phase of the disease. Occasionally, outbreaks appear due to stressful situations associated to malnutrition, concurrent infections, vaccinations, seasonal increase of vector populations, immunodepression and/or introduction of infected animals, causing high mortality. In some occasions, outbreaks in goats, sheep and cattle livestock produced by *T. vivax* have been reported in the States of Falcón and Zulia, with a prevalence of 60 and 78 %, respectively, determined through direct microscopic examination (García et al. 2000; Simoes et al. 2009) and in horses by *T. evansi* in the State of Apure, with an active infection of 5.5–12 % (García et al. 2000). Horses are essential for the management of cattle; thus, it is thought that equine trypanosomosis has an important negative impact on Venezuelan beef production (García et al. 2000; Castellanos et al. 2010; Forlano et al. 2011; Moreno et al. 2013). Recent studies in Venezuela estimated US\$ 7,486,000 in losses, because of for the lack of diagnosis and adequate treatment for trypanosomosis in horses (Moreno et al. 2013). This situation generates substantial losses in the country's livestock industry due to diminished production of milk, meat, and its derivatives, costs of treatment and animal reduction.

The diagnosis of trypanosomosis is routinely performed through parasitological techniques such as micro-haematocrit centrifugation technique (MHCT) due to their simplicity and low costs. However, this technique have low sensitivity since it is not able to detect the presence of the parasite in the chronic phase of the disease when parasitaemias are low (Desquesnes 2004; González et al. 2006; Fernández et al. 2009; Ramírez-Iglesias et al. 2011). Therefore the most suitable option for sensitive and specific trypanosomosis diagnosis is the PCR (polymerase

chain reaction) since it is allow to detect the presence of the parasite in chronically infected animals and differentiating the species involved in the infection (Dávila et al. 2003; Herrera et al. 2004; Njiru et al. 2005; Fernández et al. 2009; Ramírez-Iglesias et al. 2011; Gonzatti et al. 2014). Few studies of detection of *T. vivax* or *T. evansi* by PCR have been carried out in Venezuela, one in cattle in the State of Mérida where a 2.8 % of active infection was detected by MHCT, confirming the presence of *T. vivax* by PCR (Bolívar et al. 2006). Other studies performed in buffaloes from the States of Guárico (García et al. 2005), Falcón, Cojedes and Barinas (García et al. 2006) a prevalence of 10–20 % for *T. vivax* was determined without detecting infections by *T. evansi* and ones carried out in sheep from the Apure State where infection by *T. vivax* was confirmed in positively diagnosed samples by MHCT (4.35 %) (García et al. 2009). At present, molecular studies have not been carried out indicating the presence of *T. evansi* in cattle livestock in Venezuela. Therefore, the aim of this work was to evaluate the presence of *T. evansi* in Venezuelan cattle by PCR technique in small stockbreeding production units from the Miranda and the Aragua States.

Materials and methods

Sampling

Blood samples were taken from the cattle jugular veins and stored in tubes with anticoagulant (EDTA). A randomly representative number of cattle were sampled in a small stockbreeding production unit, with less than 100 animals. Approximately 20 % of each farm was sampled: 38 animals from 4 farms in San Casimiro, Municipality of San Casimiro, State of Aragua and 47 animals from 5 farms in “Laguneta de la Montaña”, Municipality of Guaicaipuro, State of Miranda. Sampling was carried out during the month of May at the beginning of Venezuela's rainy season.

Parasitological diagnosis by Micro-haematocrit Centrifugation Technique (MHCT)

About 75 µL of fresh blood were taken with a heparinized capillary and centrifuged for 5 min at 12,000 rpm. The tubes were examined using a light microscope (10× eyepiece and 10× objective) at 100× magnification to detect motile trypanosomes near the buffy coat (Woo 1969). Student *T* test was implemented to compare the average values of haematocrit from animals positively diagnosed for trypanosomosis with negatively diagnosed animals. Results were considered as statistically significant with values of $P < 0.01$.

Molecular diagnosis by PCR

DNA extraction from animals whole blood samples

DNA was obtained with the genomic DNA purification kit (Wizard) following the protocol from the provider (Promega®). To concentrate the parasites in the leukocyte layer, 1 mL of whole blood with anticoagulant was centrifuged at 15,000× rpm for 1 min and 300 µL of this layer were separated for DNA extraction. DNA integrity was evaluated in 0.8 % agarose gels by horizontal electrophoresis at 100 V for 30 min and visualized with transilluminator using SYBERSafe at a concentration of 1 µL/mL. DNA concentration (ng/µL) was determined by spectrophotometry at 260 nm from 2 µL of the sample through a NanoDrop™ 1000 (Thermo Scientific).

Polymerase chain reaction (PCR)

PCR was performed with 300 ng of purified DNA from cattle, ITS1 CF/BR genus-specific primers described by Njiru et al. (2005), which amplifies the sequence of intergenic spacers located between genes 18S and 5.8S of ribosomal DNA, highly conserved and represented in the genome (multi-copy locus) of different kinetoplastid species (Desquesnes et al. 2001). These primers generate amplification products of a specific size, 250 bp for positive *T. vivax* samples and 480 bp for *T. evansi*. 100 ng of DNA purified from blood of experimentally infected animals in peak of parasitemia, adult albino rats (Sprague–Dawley) for *T. evansi* and 6-months-old cross-bred sheep for *T. vivax*, were used as positive controls. The Venezuelan strains used were: *T. evansi* TeVA1 (Desquesnes 2004) and *T. vivax* TvGP1 (Gómez-Piñeres et al. 2014), which were isolated from blood samples taken from a naturally infected horse from the State of Apure, and cattle of Parmana, State of Guárico respectively. The samples were preserved in liquid nitrogen and were expanded in healthy animals (Fernández et al. 2009; Gómez-Piñeres et al. 2014). The reaction mixture for PCR was prepared in a final volume of 25 µL which contained: buffer 1X, 2 mM of MgCl₂, 200 µM of each dNTP's, 1 µM of each primer and 1.5 U of the Taq polymerase enzyme. The amplification products were analyzed by horizontal electrophoresis with 2 % agarose gels/SYBERSafe 1 µL/mL at 100 V for 1 h and visualized with UV transilluminator. Diagnosis was considered positive when a specific product looking forward, was amplified by PCR.

ITS1-PCR products of cattle diagnosed as parasitologically positive to *Trypanosoma* spp. from San Casimiro were purified (Promega PCR purification kit) and sent at least 5 times for sequencing to CeSAAN (Centro de Secuenciación y Análisis de Ácidos Nucleicos) in IVIC (Instituto Venezolano de Investigaciones Científicas).

Sequencing was performed using an Applied Biosystems semi automatic ABI3130 Analyser. The obtained ITS1 *T. evansi* sequence was aligned using MAFFT (Multiple Alignment using Fast Fourier Transform) version 7 and a percent identity matrix was produced in order to evaluate sequence similarities with others trypanosome species.

Results

Parasitologic diagnosis of cattle trypanosomosis by micro-haematocrit centrifugation technique (MHCT)

Parasitologic analysis by MHCT detected the presence of *Trypanosoma* spp. in both of the evaluated cattle populations. In the “Laguneta de la Montaña” sector in the Miranda State, 3 out of the 47 sampled animals were diagnosed as positive, with a percentage of detection of 6.4 %. On the other hand, in the San Casimiro sector, State of Aragua, 7 out of 38 of the sampled cattle were diagnosed as positive which reflects an 18.4 % of animals with active infection; these results are summarized in Table 1.

Molecular diagnosis of cattle trypanosomosis by PCR

DNAs from all of the cattle sampled were evaluated by PCR, using ITS1 genus-specific-primers to determine the real prevalence and identify the species involved in the infection. In Figs. 1 and 2, the products of amplification obtained by PCR for samples of each sector are shown. In the “Laguneta de la Montaña” sector (Fig. 1), the expected amplification product for *T. vivax* is observed in 9 animals (19 %) and for *T. evansi* in 3 animals (6.4 %), as well as double infections in 2 animals, out of 47 of the sampled bovines. In the San Casimiro sector (Fig. 2), it must be emphasized that PCR only generated the expected amplification product for *T. evansi* in 19 out of 38 sampled bovines which represents 50 % of the infected population; the obtained results are summarized in Table 1.

Sequence analysis

The presence of *T. evansi* was confirmed in parasitologically positive cattle from San Casimiro through nucleotide sequence analysis obtained from amplification products of PCR-ITS1. Table 2 shows ITS1 sequences from different isolates of *T. brucei*, *T. evansi*, *T. theileri* and *T. vivax*, from the GenBank database and identity comparison nucleotide sequences. A nucleotide sequence identity in a range of 97.7–99.53 % was obtained from *T. evansi* isolates from different countries (Egypt, China and Argentina)

Table 1 Parasitological and molecular diagnosis of bovine Trypanosomosis in small livestock units in Venezuela

Diagnosis method	Laguneta, Miranda State (N = 47)		San Casimiro, Aragua State (N = 38)	
	+	%	+	%
Parasitological, MHCT	3	6.4	7	18.4
Molecular PCR-ITS1				
<i>T. vivax</i>	9	19	0	0
<i>T. evansi</i>	3	6.4	19	50
Double infection	2	4.3	0	0
Total	14	30	19	50

(+) positive animals by each diagnosis method, (%) percentage of detection

N number of evaluated samples, *MHCT* micro-haematocrit technique, *PCR-ITS1* polymerase chain reaction using ITS1 CF/BR primers

Fig. 1 Molecular diagnosis of bovine Trypanosomosis from the “Laguneta de La Montaña” sector, Municipality of Guacaipuro, State of Miranda. Electrophoresis analysis of amplification products obtained by PCR using ITS1 primers, lanes (1–47) purified DNA of bovine blood samples; (v) *T. vivax* DNA purified from blood of experimentally infected ovine; (e) *T. evansi* DNA purified from blood of experimentally infected rats; (H) control without DNA. (STD) 100 bp marker

and hosts (camels, buffaloes and capybaras) (Amer et al. 2011; Tian et al. 2011; Eberhardt et al. 2014), and 99.31 % from *T. brucei* (Agbo et al. 2001). Additionally, this sequence had a 97.53 % of identity with ITS1-TeVA1 (data not shown), which is highly homologous to the reported sequences for Asian *T. evansi* isolated from horses. Finally, Table 2 shows a decrease in the percentage of ITS1 sequence identity when compared to other trypanosomes (*T. vivax* and *T. theileri*) present in South America (Venezuela and Brazil). The ITS1 *T. evansi* sequence isolated from Venezuelan cattle, State of Miranda, San Casimiro (ITS1-TeVSCc) has been submitted to GenBank with the accession number KR055671.

Haematocrit evaluation

Haematocrit levels in cattle from “Laguneta de la Montaña” were registered between 25 and 40 % in the studied livestock, with an average of $30.4 \% \pm 4.0$ for healthy animals and $29.1 \% \pm 1.7$ for those positively diagnosed. In the latter, an average haematocrit of $29.3 \% \pm 1.5$ was obtained for *T. vivax* infected animals, $27.7 \% \pm 2.1$ for *T. evansi* and $30.0 \% \pm 1.4$ with double infections. No significant differences were found ($P > 0.01$) between any of the haematocrit average values for animals diagnosed as positive or negative. Meanwhile in San Casimiro, lower haematocrit values were registered between 20 and 34 %

Fig. 2 Molecular diagnosis of bovine Trypanosomosis from the Municipality of “San Casimiro”, State of Aragua. Electrophoresis analysis of amplification products obtained by PCR using ITS1 primers, lanes (1–38) purified DNA of bovine blood samples; (v) *T. vivax* DNA purified from blood of experimentally infected ovine; (e) *T. evansi* DNA purified from blood of experimentally infected rats; (H) control without DNA. (STD) 100 bp marker

Table 2 Identity comparison of ITS1 Trypanosoma sequences from different isolates

<i>Trypanosoma</i>	GenBank accession numbers	TeVSCc KR055671 %
<i>T. brucei</i> (Agbo et al. 2001)	AF306775.1	99.31
<i>T. evansi</i> (Amer et al. 2011)	AB551922.1	99.31
	AB551921.1	99.31
	AB551919.1	99.31
<i>T. evansi</i> (Tian et al. 2011)	FJ712715	99.08
	FJ712713.1	99.08
<i>T. evansi</i> (Eberhardt et al. 2014)	KC988262.1	99.53
	KC988260.1	99.53
<i>T. theileri</i> (García et al. 2011)	HQ664827.1	59.93
<i>T. vivax</i> (Cortez et al. 2006)	DQ316051	39.65
<i>T. vivax</i> (Cortez et al. 2006)	DQ316045	39.67

for the evaluated herd with an average haematocrit of $28.3 \% \pm 3.5$ for healthy animals and $23.3 \% \pm 3.3$ for infected animals, amongst which lower values were registered for those parasitologically positive individuals. The determined average value for animals diagnosed as positive was significantly different ($P < 0.01$) to the one obtained for healthy animals.

Discussion

Infection in all the parasitologically positive animals was confirmed by PCR in both studied sectors. As expected, PCR was able to diagnose a higher number of animals as positive compared to MHCT (Table 1). The advantages of PCR over MHCT as a diagnostic method have been widely

demonstrated, especially during the chronic phase of animal trypanosomosis (Fernández et al. 2009). In fact, other epidemiological studies indicate that the determined prevalence with PCR is higher than that established by MHCT (Dávila et al. 2003; Herrera et al. 2004). These differences are due to sensitivity thresholds of the applied techniques. PCR has a sensitivity of 1–20 parasites/mL of blood (Desquesnes and Dávila 2002). Therefore, animals that resulted false negative by MHCT possibly had parasitaemias below the sensitivity threshold of this method, 100–500 parasites/mL (Desquesnes et al. 2013b). Low parasitaemias makes it impossible the detection and morphological differentiation by Giemsa stain blood smears, which has less sensibility than MHCT. These results confirm the superiority of PCR as the diagnosis method for cattle trypanosomosis.

The majority of studies about animal trypanosomosis by PCR have been performed using specific-primers to detect one species at a time with each reaction. Thus, the presence of *T. evansi* in South America wild stock has been described in some studies (Dávila et al. 2003; Gonzales et al. 2003; Herrera et al. 2004; Gonzales et al. 2007; Mekata et al. 2009) and the occurrence of double infections on cattle has been documented in the Pantanal region of Brazil (Dávila et al. 2003). However, even though ITS1 generally has a lower sensitivity in comparison with species-specific primers for the detection of *T. evansi* (Fernández et al. 2009) and *T. vivax* (Desquesnes and Dávila 2002; González et al. 2006), it allows the detection of double infections in only one reaction, saving time and costs. The use of these primers allowed establishing the presence of *T. vivax* and *T. evansi*, as well as double infections in the studied regions. Additionally, ITS1 primers are capable of generating amplification products of

other trypanosomes species highlighting even more its utility (Njiru et al. 2005) as occurred in a study carried out in buffaloes in the Guárico State, where the presence of a specific product of 400 bp from *T. theileri* was detected by PCR using these primers (García et al. 2006).

Our results indicate that there are differences regarding the trypanosomosis situation in both sectors. In “Laguneta de la Montaña”, using PCR gave a higher prevalence for *T. vivax* (19 %) than for *T. evansi* (6.4 %), as well as the ability to detect double infections. The low prevalence of *T. evansi* could be associated to the low pathogenicity of the parasite in cattle, which did not present clinical signs and in which cryptic parasitaemias were only detectable by PCR in the majority of animals. A similar situation was described in endemic zones in the border region of Pantanal in Brazil and Bolivia, with prevalence between 1 and 8 % of *T. evansi* and between 19 and 44 % of *T. vivax* (Dávila et al. 2003; Gonzales et al. 2007). Similarly, a low prevalence of *T. evansi* by PCR in cattle has been described in other epidemiological studies in South America, determining a prevalence between 1 and 10 % (Gonzales et al. 2003; Herrera et al. 2004) without reports of pathogenic effects (Desquesnes et al. 2013a).

Low parasitaemias reduce the risk of mechanical transmission (Dávila et al. 2003; Gonzales et al. 2007; Desquesnes et al. 2009). According to Foil et al. (1987), approximately 10 nL of blood remains in the mouth parts of tabanids after feeding, indicating that a parasitaemia of 10^5 parasites/mL would allow mechanical transmission of trypanosomes. In this sector only 3 animals resulted positive by MHCT, the rest presented undetectable parasitaemias, less than the established threshold for this method, hence mechanic transmission would be less likely under these conditions.

Haematocrit is a clinical parameter indicative of the animal's physical condition. Values of haematocrit described for healthy cattle are between 24 and 46 %, with an average of 35 % (Dávila et al. 2003). Thus, average haematocrit values determined for “Laguneta de la Montaña” cattle diagnosed as positive as well as negative can be considered as normal. Normally, anemia is related to high parasitaemias in the initial phase of the infection, if parasitaemia is low, normal haematocrit values are expected which is a characteristic of animals in a chronic state. Therefore, this situation reduces the risk of mechanical transmission amongst cattle in the “Laguneta de la Montaña” sector. This leads to consider cattle as reservoirs of low susceptibility for *T. evansi* and for themselves regarding *T. vivax*.

The epidemiological situation looks different in the San Casimiro sector. A higher prevalence of *T. evansi* was determined by MHCT (18.4 %) as well as by PCR (50 %)

and the presence of *T. vivax* was not detected (Table 1). This finding was, however, different from previous reports on the prevalence of trypanosomes in South America (Dávila and Silva 2000; Gonzales et al., 2007). In regions of Peru and Bolivia where the prevalence of *T. evansi* was higher than in *T. vivax* a similar situation was described by Mekata et al. 2009, and in the case of Pucallpa only *T. evansi* was detected (Mekata et al. 2009) This situation can be attributed to regional differences in trypanosome distribution as well as the fact that we used PCR which allows the detection of trypanosomes in even apparently aparasitemic, chronically infected animals (Mekata et al. 2009). Factors such as differences in strain virulence, host susceptibility, animals in chronic phase or even the individual nutritional status have been related to the presence of subpatent parasitaemias for *T. evansi* in enzootic areas (Herrera et al. 2004). In our case the situation described in San Casimiro fits some of those presented factors. For example, the sampling was at the beginning of the rainy season, offering a suitable environment for vector flies to thrive. In this season, an increase in the amount of haematophagous insects tends to occur in Venezuela (García et al. 2000) as has been described in different regions of Brazil (Batista et al. 2012). There seems to be a positive correlation between rainy season, the increase in vector population and an increase in prevalence of the disease (Dávila et al. 2003) as well as of the risk of trypanosomosis outbreaks (Batista et al. 2012). Although a correlation between these factors seems logical and has been established in other regions, sampling in the dry season is necessary to confirm this correlation. Likewise, the presence of animals with parasitaemias higher than 500 p/mL detected by MHCT, possibly contributes to infection transmission between cattle. According to these factors, it has been demonstrated that the incidence of transmission is directly associated to parasitaemia and vector density in the studied area (Desquesnes et al. 2009). The iatrogenic transmission caused by the use of surgical instruments or non-sterile needles is also of importance, especially during vaccination campaigns and massive treatments contributing to disease propagation (Dávila and Silva 2000; Desquesnes et al. 2013b).

Another factor to highlight was the observation of frequent bites by haematophagous bats on the evaluated animals and the high density of these mammals in this area. These mammals are crucial in the epidemiology of the disease in South America. They are simultaneously host, reservoir and vector of *T. evansi*, maintaining the presence of the parasite in their colonies in the absence of their primary host (Desquesnes 2004; Desquesnes et al. 2013b). All of these described factors possibly favored the establishment of a *T. evansi* transmission cycle, facilitating

dissemination of the infection between cattle in the San Casimiro sector.

Additionally, the animals of San Casimiro had poor body condition. Half the population, diagnosed as positive for *T. evansi* by PCR, presented a lower haematocrit to be considered as normal minimum, which was significantly different ($P < 0.01$) to the average registered for healthy animals. Despite this, it is necessary to evaluate other clinical parameters to establish the pathogenicity of the strains present in the cattle population of San Casimiro. The decline of physical conditions in these animals could be associated to a number of factors like stress, nutritional deficiencies, and the presence of other infectious agents: such as intestinal parasites or other haemotropic agents, causing weakness and low immunity favouring parasitaemia exacerbation. Moreover, blood spoliation by vampire bats is an additional reason for lower haematocrit and body condition.

The presence of *T. evansi* had never been reported in cattle livestock in Venezuela. In fact, this species is not very common on cattle in South America and there are limited reports characterized by extremely low parasitaemias in Brazil, Bolivia and Peru (Dávila et al. 2003; Herrera et al. 2004). The absence or presence of a pathological state caused by *T. evansi* could be related to susceptibility or adjustment of the herd to the region and/or the pathogenicity of the circulating isolate. Differences in disease severity caused by *T. evansi* have been described depending on the host, strain virulence and geographic region (Desquesnes et al. 2013a). While *T. evansi* has a lower pathogenicity in cattle and buffalo livestock in South America, in the Asian continent (Philippines, Thailand and Vietnam) there are isolates that can induce anemia, hyperthermia, weight loss, diminished production of meat and milk, damage to the nervous system and abortions (Desquesnes et al. 2013a). However, is well documented that trypanosomosis in cattle induce alterations in the host's defense system and compromises the ability of animals to control this disease, as well as secondary infections (Taylor and Mertens 1999, Namangala 2011). These parasites activate, modulate and control many aspects of the host natural and adaptive immunity during infection and can modulate the host responses to evade immune killing to survive in infected host and hence allow transmission to the next host (Namangala 2011). Therefore, infections by *T. evansi* could provide an adequate environment in cattle increasing its susceptibility to mixed infections. It would be interesting to study cattle and buffalo susceptibility to different geographical isolates of *T. evansi* in Venezuela. The possible introduction or inducement of new genotypes of *T. evansi* with unknown virulence could trigger outbreaks of this disease.

All species of mammals can be infected with *T. evansi* but the principal reservoirs vary geographically. Many studies have suggested that variation in the transferrin receptor-binding region of trypanosome is one of the factors contributing to the difference in the pathogenicity of parasites and in the ability to infect a wide range of host species (Mekata et al. 2009). It would be interesting to analyze the diversity in the ESAG 6 and 7 genes among *T. evansi* isolates from Venezuela and compare them to previously reported variants from South America and other continents in order to determine the extent of genetic diversity and their possible relationship with the host and reservoir.

It is of importance to highlight the role that cattle may play as reservoirs for the maintenance of *T. evansi* in the environment. In Venezuela, horses (susceptible hosts) are essential for the management of cattle (bovines and buffaloes). As a result, these animals are in close contact where horses (susceptible hosts) coexist with cattle (low susceptibility reservoirs) and wild animals (reservoirs). This determines the numeric relation between horses and cattle with an $r = 0.93$, which is relatively constant in the whole country (Moreno et al. 2013). This interaction could contribute to the dynamics and maintenance of trypanosomosis in the studied areas, which makes necessary the application of treatments for the effective control of the disease preventing the appearance of outbreaks in susceptible hosts.

Until now failure to detect *T. evansi* in Venezuelan cattle might be due to the asymptomatic character of infected animals and the lack of PCR studies to detect this species. In general, PCR has been implemented as a tool for confirmation of the causal agent and to a lesser extent, for epidemiological studies. Therefore, it is recommended to pursue epidemiological studies in the country using ITS1 primers to establish the real trypanosomosis situation, its hosts and specifically to determine the agents involved in the infection. It could also be used to study the different circulating isolates found in Venezuela in a more thorough level, including the interaction of *T. evansi* with its vectors and reservoirs.

Conclusions

The presence of *T. evansi* in Venezuelan cattle was first reported in this study. This result is based on positive molecular diagnosis by PCR using the primer ITS1 and on the high percentage of sequence identity with other reported ITS1 obtained for *T. evansi* in different hosts and geographical areas. The presence of this parasite in cattle could play an important role in the maintenance of the infection in animal production units. The coexistence of

vectors, reservoirs and susceptible hosts, favors the interaction throughout the *T. evansi* epidemiological chain, facilitating its dissemination amongst different groups of susceptible hosts such as horses. This situation has determined the endemic character and appearance of occasional outbreaks of the disease in the country. Therefore, it is necessary to accomplish epidemiological studies for a sensitive and specific diagnosis of trypanosomosis with the purpose of taking adequate sanitary measures for the control of the disease and avoid economic losses in the livestock field.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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