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ARTICLE

Biochemical and physiological parameters associated with *Trypanosoma evansi* prevalence in wild capybaras (*Hydrochoerus hydrochaeris*)

A.T. Eberhardt, P.M. Beldomenico, L.D. Monje, and A.L. Racca

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Abstract: Parasites can be detrimental to the health of wildlife populations and may negatively affect several aspects of the life history of their hosts. Investigating host health, therefore, is key to understanding important mechanisms of the host–parasite interaction at the individual and population levels. Recently, we reported a prevalence of 10% of *Trypanosoma evansi* Steel, 1884 in a population of capybaras (*Hydrochoerus hydrochaeris* (L., 1766)) from Esteros del Iberá, Argentina; however, the impact of *T. evansi* infection on capybaras is unknown. The aim of this study was to explore associations between *T. evansi* infection and biochemical and physiological parameters in wild capybaras using blood samples (n = 60) from a managed population of free-ranging capybaras from Esteros del Iberá. Infection by *T. evansi* was negatively associated with body condition, albumin, alpha-2 globulin concentrations, albumin/globulin ratio, and eosinophil counts, and it was positively associated with spleen index and gamma-globulin concentrations. These results suggest that *T. evansi* infection may pose a significant impact on the health of wild capybaras.

Key words: haematology, *Hydrochoerus hydrochaeris*, serum proteinogram, *Trypanosoma evansi*.

Résumé : Les parasites peuvent être néfastes pour la santé des populations d'animaux sauvages et avoir une incidence négative sur plusieurs aspects du cycle biologique de leurs hôtes. L'étude de la santé des hôtes revêt donc une importance clé pour la compréhension des mécanismes qui jouent un rôle dans les interactions entre hôtes et parasites à l'échelle de l'individu et de la population. Nous avons récemment fait état d'une prévalence de 10 % de *Trypanosoma evansi* Steel, 1884 dans une population de capybaras (*Hydrochoerus hydrochaeris* (L., 1766)) des Esteros del Iberá (Argentine); toutefois, l'impact de l'infection à *T. evansi* chez les capybaras n'est pas connu. La présente étude visait donc à examiner les associations entre l'infection à *T. evansi* et des paramètres biochimiques et physiologiques chez les capybaras sauvages en utilisant des échantillons sanguins (n = 60) prélevés dans une population gérée de capybaras en liberté des Esteros del Iberá. L'infection à *T. evansi* présentait une association négative avec l'embonpoint, les concentrations d'albumine et d'alpha-2 globulines, le rapport albumine/globuline et les comptes d'éosinophiles, et une association positive avec l'indice splénique et les concentrations de gammaglobulines. Ces résultats donnent à penser que l'infection à *T. evansi* pourrait avoir un impact significatif sur la santé des capybaras sauvages. [Traduit par la Rédaction]

Mots-clés : hématologie, *Hydrochoerus hydrochaeris*, protéinogramme sérique, *Trypanosoma evansi*.

Introduction

Parasites can be detrimental to the health of wild mammalian populations and may negatively affect several aspects of the life history of their hosts (Gulland 1995), which may ultimately drive host populations to drastic declines or even extinctions (Tompkins et al. 2011). Investigating host health, therefore, is essential to better understand mechanisms of the host–parasite interaction at the individual and population levels. Recently, we have reported biochemical and physiological parameters of free-ranging capybaras (*Hydrochoerus hydrochaeris* (L., 1766)), which may provide useful information to evaluate their health status and their life history (Eberhardt et al. 2016).

The capybara is the large rodent inhabiting tropical, subtropical, and temperate freshwater wetlands of South America (Moreira et al. 2013). It is one of the most intensely used wildlife species in South America owing to the value of its hide and also because it provides an additional source of protein for many local communities (Bolkovic et al. 2006; Moreira et al. 2013). This species is host to a very rich parasite community, including several specific helminths and protozoans that show high prevalence and ubiquity (Moreno et al. 2013) and haemoprotozoan parasites like *Trypanosoma evansi* Steel, 1884 (Morales et al. 1976). In South America, capybaras are regarded as one of the main reservoir hosts of *T. evansi* and therefore deemed a major source of infection for domestic animals. In some places like in Esteros del Iberá (Corrientes Province, northeastern Argentina), capybaras are present at high densities, living close to farms and urban settlements, resulting in an extensive human–domestic–wildlife interface that may represent a potential risk to public health and animal husbandry.

Trypanosoma evansi is a haemoprotozoan parasite, with a broad host range (found in nine orders of mammalian species), that is

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widely distributed in tropical and subtropical regions of the world (Herrera et al. 2004). In South America, *T. evansi* is found in host species introduced by humans, such as horses (*Equus caballus* L., 1758), cattle (*Bos taurus* L, 1758), buffaloes, sheep (*Ovis aries* L., 1758), and goats (*Capra hircus* L., 1758), and also in a large range of wild hosts, including South American coatis (*Nasua nasua* (L., 1766)), white-lipped peccaries (*Tayassu pecari* (Link, 1795)), small rodents, and capybaras (Herrera et al. 2004; Rademarker et al. 2009; Eberhardt et al. 2014). In this region, the natural transmission of the parasite occurs mainly by mechanical transmission via arthropod vectors, mainly species of the genus *Tabanus* L., 1758, and via vampire bats (*Desmodus rotundus* (E. Geoffroy, 1810)) (Hoare 1965).

Although infections by *T. evansi* have been reported in many South American wild hosts, only in coatis has it been found to cause a measurable impact on the host's health, i.e., anemia, emaciation, and immune suppression (Herrera et al. 2004; Olifiers et al. 2015). In infected capybaras, there is no evidence of pathological effects despite high levels of parasitemia, suggesting that this host species could act as a reservoir for *T. evansi* in the region (Franke et al. 1994; Arias et al. 1997; Herrera et al. 2004).

The determination of biochemical and haematological parameters is routinely used in human and veterinary medicine to assess the health of an individual (Kaneko 1997). For example, variations in concentrations of various types of blood cells, as well as changes in their morphology, are indicative of particular physiological or infectious status. It has been demonstrated that red blood cells (RBC) and lymphocytes may be important indicators of condition and fitness (Beldomenico et al. 2008). Decreases in RBC (anaemia) or in lymphocyte levels (lymphopaenia) are detrimental effects observed during infections by many pathogens (e.g., Herrera et al. 2004; Beldomenico et al. 2009; Olifiers et al. 2015). In this regard, to date the only health parameter reported in capybaras infected with *T. evansi* is the haematocrit value (i.e., the proportion of the blood that is composed of RBC and that is decreased in anaemic individuals) (Herrera et al. 2004). Total protein and protein fractions are also frequently used to assess the health of an individual (Weiss and Wardrop 2010). We have previously reported these fractions in capybara, identifying five fractions of serum proteins (Eberhardt et al. 2016). Establishing alterations in the pattern of these fractions may be useful information when interpreted concomitantly with other clinical and laboratory findings (Thomas 2000). Nevertheless, all these parameters in wildlife could be influenced by a myriad of factors (e.g., seasonal changes, competition, population density, resource availability), and therefore, its interpretation requires caution. It is necessary to establish baseline values for a given species to be able to interpret the information they provide. Recently, we reported a prevalence of 10% of *T. evansi* in a wild capybara population from Esteros del Iberá, Corrientes, Argentina (Eberhardt et al. 2014). Herein we expand that study to establish the presence of associations between *T. evansi* infection and biochemical and physiological parameters.

Materials and methods

Study area

The ranch "Rincón del Socorro", Corrientes Province, Argentina (28°36′S, 57°49′W), is a 12 000 ha natural private reserve within the macrosystem Esteros del Iberá. This ecosystem is dominated by swamps and marshlands connected by an extensive system of shallow lakes. The climate is subtropical and humid without a dry season. However, in spring (October–December) and summer (January–March), both rainfall and temperature are higher than during the rest of the year. Mean minimum temperature is 16–17 °C in June and July (winter) and mean maximum temperature is 27–28 °C in January and February (summer). The range spans from −5 to 44 °C during the year. The cumulative precipitation recorded by a meteorological station set near the ranch was 1487 mm in 2010, 1280 mm in 2011, and 1426 mm in 2012 (Red Iberá – Entidad Binacional Yacyretá).

Sample collection

Sixty samples were obtained from a population of free-ranging capybaras from the ranch Rincón del Socorro. Over a 2-year period (August 2010 – September 2012), monthly samples were collected from capybaras that were euthanized by gunshot without prior capture, removed from the population as part of a program to limit overpopulation (three per month) that was authorized by the Dirección de Recursos Naturales of Corrientes Province. The animals were all adults except one, which was a subadult according to its body size. Thirty-two were males and 28 were females, and weighed from 27 to 70 kg (mean = 55.13 kg). Blood samples were taken immediately after euthanasia from the cava vein and stored in tubes with and without an anticoagulant (5 mmol/L EDTA). Samples with anticoagulant were kept refrigerated (4 °C) and processed within 6 h of collection. Samples without anticoagulant were immediately allowed to clot at room temperature and then centrifuged (1500*g* for 10 min), and both serum and blood clot were aliquoted into labeled microtubes, transported in liquid nitrogen to the laboratory, and then stored at –20 °C until further processing.

Haematological and biochemical parameters

Samples with anticoagulant were used to produce blood smears and blood cell counts using improved Neubauer's chambers, as described in Eberhardt et al. (2016). Smears were fixed and stained with May–Grünwald–Giemsa and 200 cells were counted.

Serum samples were used to determine total protein and albumin/globulin (A/G) ratio by colorimetric assays using an automated biochemical analyzer (Wiener Lab. Group, Santa Fe, Argentina) and protein fractions by electrophoresis followed by quantified in an automated densitometer (Interlab SRL, Rome, Italy), as described in Eberhardt et al. (2016).

Spleen index

At necropsy, the entire spleen was dissected and weighed using a digital scale (Ohaus Traveller, precision 0.01 g). The spleen index was estimated by dividing the spleen mass by body mass and multiplying by 100 (Eberhardt et al. 2013).

Detection of *T. evansi* infection

The intensity of *T. evansi* infection in the blood was evaluated by count of trypanosomes in blood smears and by real-time polymerase chain reaction (PCR) from blood clots (see details in Eberhardt et al. 2014).

Body condition

Immediately after being euthanized, the animals were weighed (body mass) using a mechanical scale (precision 0.2 kg), measured (the morphometric measure used in this study was total length from nose to tail, precision 0.5 cm), and then dissected. The measure of body condition was a residual index that was estimated with a linear regression of body mass against total length, which included adjustment by pregnancy status (three-level factor: nonpregnant (which includes males), early pregnancy, and advanced pregnancy; the model is mass ~ total length + pregnancy status, where the coefficients for length was 1.005; $p < 0.001$, $R^2 = 0.796$) (Eberhardt et al. 2016). We took into account pregnancy status because in a female with advanced pregnancy, a substantial proportion of its body mass corresponds to the placenta and its contents, which does not directly reflect the body condition of a female.

Ethical considerations

All the procedures were performed according to the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (Federation of Animal Science Societies 2010) and the protocol was

Table 1. Parameters of health (including biochemical and physiological parameters, body condition, body mass, and spleen mass) of uninfected and infected capybaras (*Hydrochoerus hydrochaeris*) from Esteros del Iberá, Argentina, with *Trypanosoma evansi*.

	Uninfected animals		Infected animals		
Parameters	Mean	Range	Mean	Range	*p*
Body mass (g)	55.94	27–70	48.08	36–62	0.0365
Body condition	0.64	–9.06 to 6.67	–4.18	–15.5 to 4.46	0.0245
Total protein (g/dL)	6.49	4.26–7.90	6.66	5.24–7.8	0.6633
Albumin (g/dL)	3.03	2.2–4	2.45	2.2–2.7	0.0018
Albumin/globulin ratio	0.81	0.52–1.25	0.65	0.48–0.92	0.0125
Alpha-1 globulin (g/dL)	0.06	0–0.1	0.05	0–0.1	0.3669
Alpha-2 globulin (g/dL)	0.81	0.3–1.2	0.64	0.25–0.9	0.0195
Beta globulin (g/dL)	1.08	0.5–2	0.9	0.68–1.2	0.0564
Gamma globulin (g/dL)	1.58	0.8–2.9	2.65	1.1–3.7	0.0128
Red blood cells (millions of cells/μL)	4.99	1.82–12.38	4.04	1.46–6.96	0.0630
Leukocytes (thousands of cells/μL)	8.13	1.6–18.6	5.53	2.5–11.1	0.0615
Lymphocytes (thousands of cells/μL)	5.85	1.31–15.9	4.47	1.26–9.42	0.1322
Neutrophils (thousands of cells/μL)	1.46	0.06–4.6	0.68	0.153–1.23	0.0519
Eosinophils (cells/μL)	403	16–1201	154	15.2–334	0.0146
Basophils (cells/μL)	54.7	0–283.5	27.38	0–55.75	0.5725
Monocytes (cells/μL)	274.54	0–1165	186.9	15.2–464.8	0.2862
Kurloff cells (cells/μL)	78.9	0–904	13.83	0–42.25	0.1514
Spleen index	201.05	92–297	273.33	206–367	0.0027

Note: Range refers to the range of values obtained in this study and *p* values are from Mann–Whitney tests.

approved by the ethics and safety committee of the Facultad de Ciencias Veterinarias of the Universidad Nacional del Litoral (Santa Fe, Argentina) under protocol No. 36/09.

Statistical analysis

Detailed descriptive statistics are offered for each variable. We used the Mann–Whitney test to compare parameters of health (including biochemical and physiological parameters, body condition, and spleen index) between infected and uninfected wild capybaras with *T. evansi*. We used the statistical software R version 3.2.4 (R Core Team 2016).

Results

Six individuals (2 females and 4 males) out of the 60 analysed tested positive for *T. evansi* using microscopy and also real-time PCR assays. A remarkable agreement between the results yielded by both methods was observed (κ = 1). The remaining 54 individuals (27 capybaras of each sex) were negative by both methods. This represents an overall *T. evansi* prevalence estimate of 10% in this free-ranging population (see Eberhardt et al. 2014).

The descriptive statistics and significance of the parameters of health measured by *T. evansi* infection status are shown in T1 Table 1.

Parameters of capybara health affected by *T. evansi* infection

Body condition

Capybaras positive for *T. evansi* by microscopy and PCR had lower body condition than those ~~which~~ tested negative using both F1 methods (W = 238, p = 0.024; Table 1, Fig. 1).

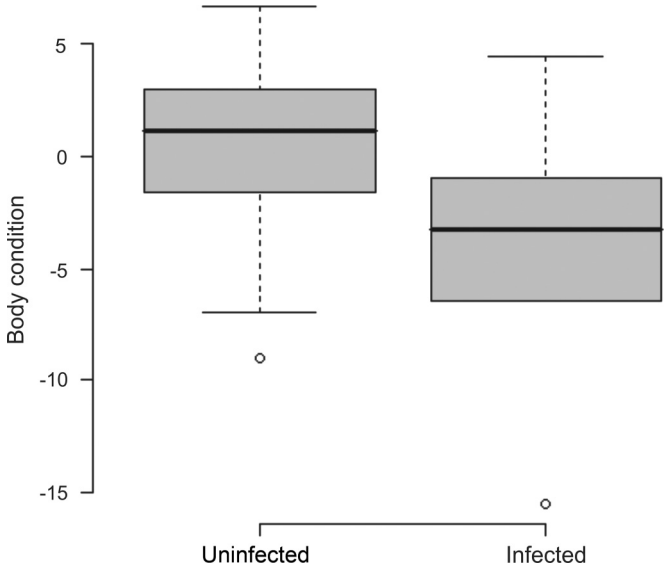
Serum proteins

The total protein, albumin, and globulin fraction concentrations for infected and uninfected individuals are presented in F2-F3 Table 1 and Figs. 2 and 3. We found that infected capybaras showed significantly lower levels of albumin (W = 265, p = 0.0018) and alpha-2 fraction (W = 231.5, p = 0.0195; Fig. 2) and higher gamma globulins (W = 67, p = 0.0195; Fig. 3) than ~~uninfectedcapybaras~~. The A/G ratio was lower in infected capybaras (W = 230.5, p = 0.0125) compared with uninfected individuals (Fig. 2).

Haematological parameters

A significant difference was observed in eosinophil counts (W = 246.5, p = 0.0145), which was lower in infected individuals (Fig. 2).

Fig. 1. Box plot showing the body condition of capybaras (*Hydrochoerus hydrochaeris*) relative to *Trypanosoma evansi* infection status. Box plots depict the median (thick horizontal line), 25%–75% quartiles (box), 10%–90% quantiles (whiskers), and outliers ~~(points)~~.



The mean was 2.62 times higher in uninfected individuals. In addition, there was a pronounced trend of infected individuals that had lower neutrophils than uninfected ones (W = 237, p = 0.052). No differences between infected and uninfected capybaras were observed for red blood cells, lymphocytes, monocytes, and Kurloff cells (Table 1).

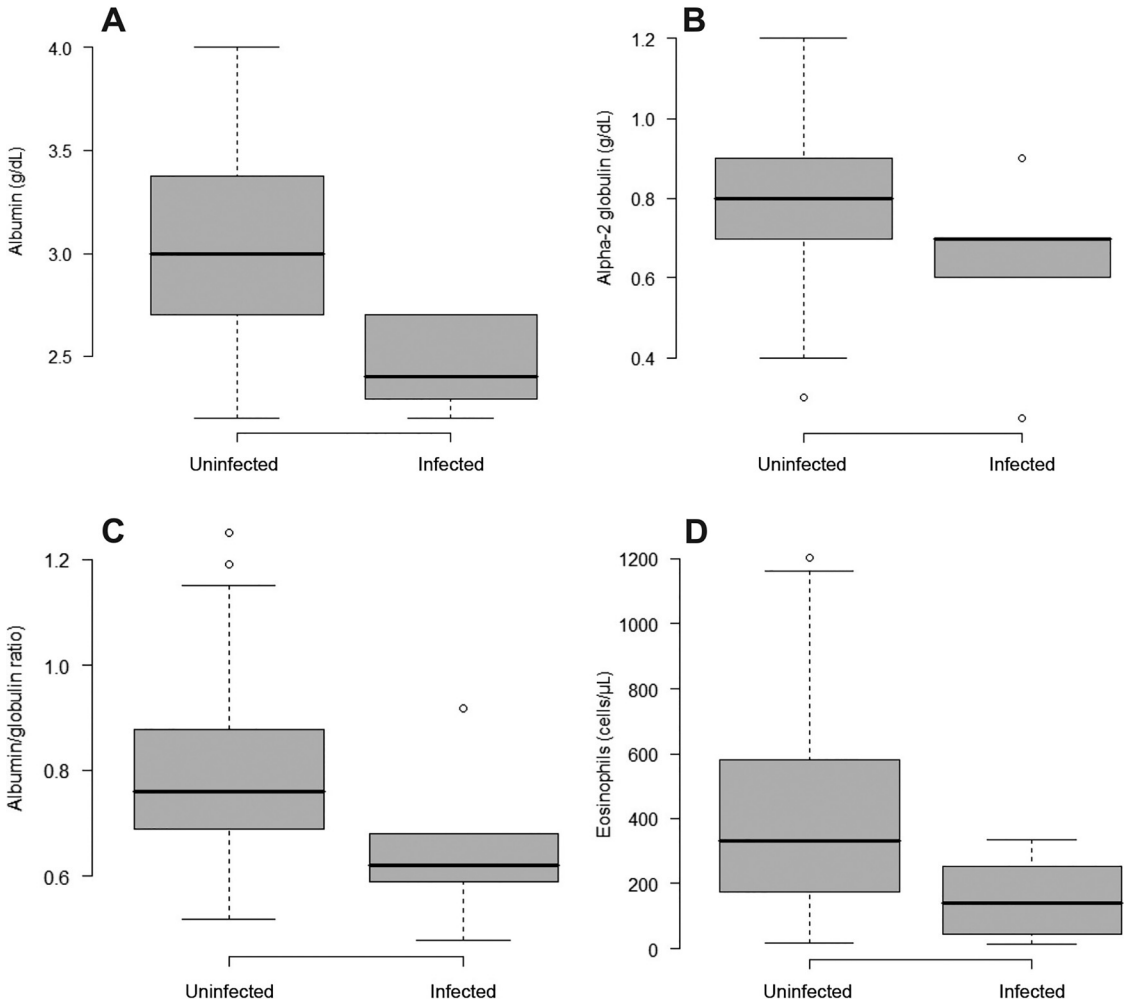
Spleen index

There was a positive association between infected animals and spleen index (W = 47.5, p = 0.002). The mean spleen index value was 36% higher in infected capybaras than in uninfected capybaras (Table 1, Fig. 3).

Discussion

Capybaras have the potential to play a relevant role in the epidemiology of *T. evansi* in South America. However, their true con-

Fig. 2. Box plots showing comparison between *Trypanosoma evansi* infected and uninfected wild capybaras (*Hydrochoerus hydrochaeris*): (A) albumin concentration (g/dL), (B) alpha-2 globulin concentration (g/dL), (C) albumin/globulin ratio, and (D) eosinophil concentration (cells/μL). Box plots depict the median (thick horizontal line), 25%–75% quartiles (box), 10%–90% quantiles (whiskers), and outliers (~~points~~).



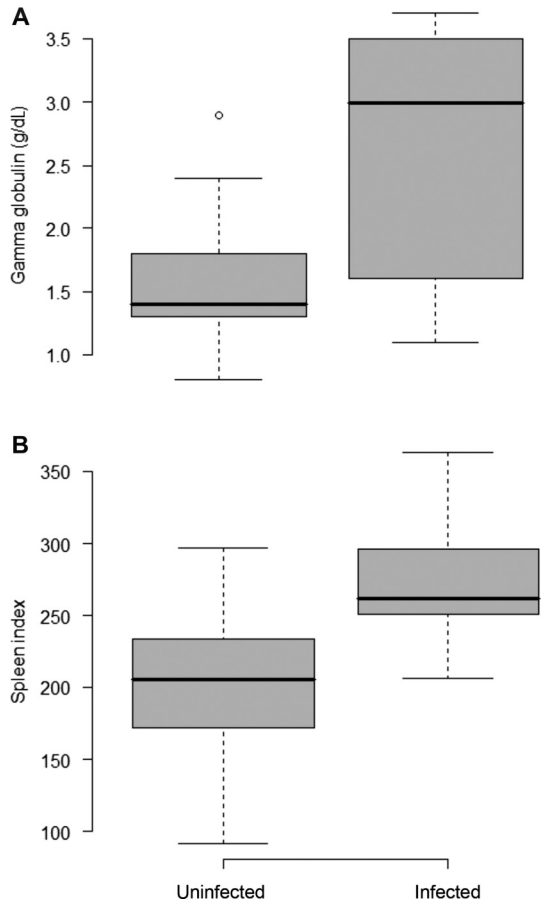
tribution remains unclear. In this regard, the aim of this study was to shed light on the relationship between host health and infection by *T. evansi* under natural circumstances, sampling a free-ranging capybara population from Esteros del Iberá, Argentina. The main findings reported here were that the infection by *T. evansi* was significantly associated with body condition, albumin and alpha-2 globulin concentrations, A/G ratio, and eosinophil counts in a negative way, but positively associated with spleen index and gamma-globulin concentration.

Diagnosis of *T. evansi* infection by conventional parasitological techniques (e.g., count of trypanosomes in blood smears) is satisfactory in animals with acute or subacute infection when trypanosomes are present in large numbers in the peripheral blood, but it is often more difficult in chronic or latent infections when parasitemia may be intermittent or very low (Aquino et al. 1999). Notwithstanding, it has been reported that real-time PCR is sensitive enough to detect low levels of parasitemia during incubation or chronic phases of the disease (Duvallet et al. 1999). Our results showed that six capybaras tested positive for *T. evansi* by both smear microscopy and real-time PCR. Given the agreement between microscopic exam of smears and real-time PCR (Eberhardt et al. 2014), these individuals might be in the acute or subacute phase of the disease. So, our interpretations about associations between health parameters and *T. evansi* infection found in this study are made assuming that we were in the presence of acute or subacute infection.

Haematological profiles respond to parasite infection as a direct result of parasite-induced blood and energy losses, upregulation of host immunity in response to infection, and even the repair of collateral damage caused by host immune mediators (Colditz 2008). Thus, examining the biochemical and physiological parameters studied herein may provide an integrated measure of the effect of parasites on hosts.

Some alterations in blood biochemistry have been reported in domestic and wild animals infected with *T. evansi* from South America. Hyperglobulinemia accompanied by hypoalbuminemia (i.e., low A/G ratio) were found in both experimental (cats (*Felis catus* L., 1758), coatis, horses, guinea pigs (*Cavia porcellus* (L., 1758)), dogs (*Canis familiaris* L., 1758), donkeys (*Equus asinus* L., 1758)) and natural (horses, coatis) *T. evansi* infections (i.e., Monzon and Villavicencio 1990; Soodan et al. 1996; Aquino et al. 2002; Herrera et al. 2002), but none of these reports discriminated protein fractions. Here we reported significant decrease in albumin concentration and A/G ratio in the blood of infected capybaras, as well as notably reduced alpha-2 fraction levels and increased levels of gamma globulins. Similar results were observed in guinea pigs and horses infected with *T. evansi*, but there were no reports of changes in alpha-globulin levels in either animal (Monzon et al.

Fig. 3. Box plot showing parameters of health in wild capybaras (*Hydrochoerus hydrochaeris*) relative to *Trypanosoma evansi* infection status: (A) gamma globulin concentration (g/dL) and (B) spleen index. Box plots depict the median (thick horizontal line), 25%–75% quartiles (box), 10%–90% quantiles (whiskers), and outliers ~~(points)~~.



1990). Notwithstanding, Verma and Gautan (1977) described a decrease in this fraction in cattle infected with this parasite. Although there are no other reports regarding all components of the alpha-2 fraction in capybaras, it is known that the main components in other rodents are the proteins haptoglobin, ceruloplasmin, and α2-macroglobulin (Kaneko 1997). These plasmatic proteins are produced by the liver, and α2-macroglobulin is a major non-immunoglobulin component (Kaneko 1997). Albumin is also produced by the liver and has a key metabolic function as a transport protein. It is also the main component of blood oncotic pressure. The decrease in albumin levels may due to (*i*) a compensatory mechanism for the maintenance of plasma osmolality increased by high globulin levels (as a result of immune response to *T. evansi*, see below) (Aquino et al. 2002) or (*ii*) deposition of high levels of systemic antigen–antibody immune complexes in the liver (among other organs), which in turn may possibly play a role in tissue damage (Tizard 2009). Although, our study did not include liver histopathology, Herrera et al. (2002) reported that coati experimentally infected with *T. evansi* presented varying degrees of liver necrosis with associated inflammation. Therefore, a disrupted functioning of the liver might also explain the decrease in plasmatic albumin and probably also in the alpha-2 fraction.

Moreover, changes in albumin, alpha-globulin, and gamma-globulin levels reported here emphasize the importance of analyzing the different protein fractions when there is a need to study the effects of trypanosomes or other pathogens on blood chemistries.

Due to the lack of commercial antibodies for capybaras, we used gamma-globulin fraction as a proxy for humoral immune response, because this fraction is essentially constituted by circulating antibodies, preferentially those produced by acquired immune response (Kaneko 1997). In this study, infected capybaras showed higher levels of gamma globulins than uninfected capybaras. There are few reports about specific antibody levels in free-ranging capybara naturally infected with *T. evansi*. (Franke et al. 1994; Arias et al. 1997; Da Silva et al. 2016). Aquino et al. (1999) have reported that specific antibody titers (anti *T. evansi*) in dogs increased progressively and remained high throughout the experiment; however, their levels were not correlated with the ability to control parasitemia. We also observed that infected capybaras had a higher spleen index than uninfected animals. Similar results were reported in mice (*Mus musculus* L., 1758) experimentally infected with *T. evansi* (Cañas et al. 1986). The fact that infected capybaras had higher spleen index and increased gamma-globulin levels than uninfected capybaras suggest a strong immune response against *T. evansi*. It has been proposed that splenomegaly might be indicative of a good immunological response to *T. evansi* infection (Tizard 2009). On the other hand, considering that the spleen is one of the main organs in which immune complexes could be depurated (Tizard 2009), the enlargement of this organ may result from the higher demand for immune complex depuration.

Anemia is the main outcome of *T. evansi* infection. There is experimental evidence in mice, brown rats (*Rattus norvegicus* (Berkenhout, 1769)), dogs, donkeys, horses, and coatis that *T. evansi* cause anemia following the first wave of parasitemia (Aquino et al. 2002; Herrera et al. 2002). In dogs, horses, and free-living animals such as coati, lower RBC were reported in animals with high parasitemia (Herrera et al. 2004; Olifiers et al. 2015). Similarly, capybaras infected with *T. evansi* showed lower mean RBC counts than uninfected animals; however, this difference was not significant ($p = 0.063$). This lack of statistical significance could result from poor statistical power, owing to the low number of capybaras infected with *T. evansi* that were reported here. However, Herrera et al. (2004) reported that capybaras naturally infected with *T. evansi* showed no difference in haematrocrit compared with uninfected capybaras. So, this capacity to control the development of anemia by capybaras could suggest a degree of tolerance to trypanosome infection. Trail et al. (1990) suggested the same tolerance to *Trypanosoma congolense* Broden, 1904 and *Trypanosoma vivax* Ziemann, 1905 in N'Dama cattle systems. The reasons underlying this tolerance in capybaras are unknown; it is noteworthy that this species and *T. evansi* do not have a long history of co-evolution, since the parasite was introduced to South America during the sixteenth century by Spanish settlers (Santos et al. 1992).

Haematological values in uninfected individuals were within ranges previously reported (Madella et al. 2006; Corriale et al. 2013). In this study, all leukocyte populations were lower in infected capybaras than in uninfected capybaras, although eosinophils were the only leukocytes that showed significantly different counts.

Previous results on leukocytes and counts of each cell type, both in naturally and experimentally infected mammal hosts, have been varied. For example, Olifiers et al. (2015) reported that coatis naturally infected with *T. evansi* also showed a reduced number of eosinophils and neutrophils; dogs showed a decrease in circulating leukocyte counts during infection (Aquino et al. 2002), whereas cats, horses, and guinea pigs exhibited an increase in circulating leukocyte counts (Monzón et al. 1991; Silva et al. 2010). Our results suggest a cellular immunological debilitation (since all leukocyte populations tended to be lower in infected individuals) at the expense of antibody production (infected capybaras showed higher levels of gamma globulins than uninfected capybaras, see above). It has been suggested that trypanosome induce antibody production specific to highly variable surface glycopro-

teins (Desquesnes et al. 2013). This is in accordance with Aquino et al. (1999), as discussed above, who showed that high specific antibody levels were not correlated with the ability to control parasitemia. This might reflect a strategy of *T. evansi* to evade the immune system of capybaras.

Finally, Olifiers et al. (2015) reported that body condition was lower in wild coatis with high parasitemia of *T. evansi* than those which tested negative, especially during the reproductive season. This is in agreement with our observations, but it should be noted that our cross-sectional results cannot distinguish cause from effect (i.e., if poor body condition is the cause or the consequence of the high intensity of infection).

All the above suggests that *T. evansi* infection is associated with deteriorated health status of capybaras, as measured by biochemical and physiological parameters. In addition, the positive association with gamma-globulin values and spleen index strongly suggests a humoural response to *T. evansi* infection. Considering that the immune response is costly (Schmid-Hempel and Ebert 2003), it could be one of the causes of the concomitant decrease in body condition (Lochmiller and Deerenberg 2000; Derting and Compton 2003; Eberhardt et al. 2013). Furthermore, capybaras have high prevalence and intensity of several specific helminths and protozoans (Moreno et al. 2013). In this way, the observed reduction in body condition might be not only the effect of *T. evansi* but also of infections by other parasites that could be more likely and severe in *T. evansi* infected individuals (Beldomenico and Begon 2010). Moreover, it should be acknowledged that infection by trypanosomes might be more likely and more severe in individuals that were a priori in a deteriorated condition (Beldomenico et al. 2009), and therefore the association found might be indicating that individuals in poor condition were more prone to become infected, and not necessarily that the deterioration was caused by the infection. Further research should provide additional detail on the patterns presented here to assess the health impact of *T. evansi* on capybaras and expand the use of biochemical and haematological parameters on free-living wildlife populations.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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