Parasitology

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Infection by *Trypanosoma cruzi* in the central nervous system in non-human mammals: a systematic review

Evaristo Villalba-Alemán[1], Mariáurea Matias Sarandy[1], Mônica Morais-Santos[1], Rômulo Dias Novaes[2] and Reggiani Vilela Gonçalves[1]

[1]Department of Animal Biology, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil and [2]Department of Structural Biology, Federal University of Alfenas, Alfenas, Minas Gerais, Brazil

Review

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Author for correspondence:
Vilela Gonçalves, E-mail: reggiani.goncalves@ufv.br

Abstract

Currently, the types and distribution of the lesions induced in the central nervous system (CNS) by *Trypanosoma cruzi* remain unclear as the available evidence is based on fragmented data. Therefore, we developed a systematic review to analyse the main characteristics of the CNS lesions in non-human hosts infected. From a structured search on the PubMed/Medline and Scopus platforms, 32 studies were retrieved, subjected to data extraction and methodological bias analysis. Our results show that the most frequent alterations in the CNS are the presence of different forms of *T. cruzi* and intense lymphocytes infiltrates. The encephalon is the main target of *T. cruzi*, and inflammatory changes in the CNS are more frequent and severe in the acute phase of infection. The parasite's genotype and phenotype are associated with the tropism and severity of the CNS lesions. The methodological limitations found in the studies were divergences in inoculation pathways, under-reporting of animal age and weight, sample calculation strategies and histopathological characterization. Since the changes were dependent on the pathogenicity and virulence of the *T. cruzi* strains, the genotype and phenotype characterization of the parasite are extremely relevant to predict changes in the CNS and the neurological manifestations associated with Chagas' disease.

Introduction

Chagas' disease is a neglected tropical infection caused by the protozoan parasite *Trypanosoma cruzi* (Chagas, 1909). Recent estimates indicate that 8 million people are infected with this parasite worldwide (WHO, 2017). This disease is closely related to poverty and is endemic in South and Central America where it is considered a public health problem with more than 10 000 deaths per year (WHO, 2017). However, due to the intense migration of *T. cruzi*-infected Latin Americans to Asia, Europe and Oceania, there has been an increase in the number of cases of Chagas' disease in these non-endemic areas since the early 1990s with successive increases in the number of cases in later years (Schmunis, 2007).

The natural route of infection of the obligate intracellular parasite *T. cruzi* occurs when a triatomine insect vector deposits infective metacyclic trypomastigotes with their feces and urine on the host's skin during blood meal (Guimarães-Pinto *et al.*, 2018). In addition to humans, *T. cruzi* infects a wide variety of domestic and wild mammals such as Carnivora, Chiroptera, Didelphidomorphia, Lagomorpha, Perissodactyla, Pilosa, Prieta and Rodentia (Añez *et al.*, 2009; Herrera, 2010), with dogs being the main domestic reservoir (Montenegro *et al.*, 2002). In addition to vector insects, transmission of parasites can also occur through non-vector pathways such as blood transfusions (Moraes-Souza and Ferreira-Silva, 2011), transplants of infected organs (Márquez *et al.*, 2013), vertical transmission (Barrios *et al.*, 2015), laboratory accidents (Dias, 2006) and by the ingestion of food contaminated with the infective forms (trypomastigotes) of *T. cruzi* (Shikanai-Yasuda and Carvalho, 2012; Domingues *et al.*, 2015). Vector transmission is mainly mediated by insects of the genus *Triatoma*, *Panstrongylus* and *Rhodnius* (Hemiptera; Reduviidae) (Coura and Viñas, 2010).

Trypanosoma cruzi is a parasite of high genetic diversity, composed of a set of strains or isolates that circulate between insect vectors and mammalian hosts (Rassi *et al.*, 2010). Although controversial, this heterogeneity has been associated with the wide variability of clinical manifestations and the different profiles of morbidity and mortality of Chagas' disease (Macedo *et al.*, 2004; Manoel-Caetano and Silva, 2007). Regarding the *T. cruzi* strains, the most recent classification describe at least six genetic lineages or discrete typing units (DTUs), named TcI to TcVI (Zingales *et al.*, 2009; Zingales, 2018). TcI predominates in the wild transmission cycle, is less resistant to antiparasitic reference chemotherapy (benznidazole and nifurtimox), and is associated with the human disease occurring in the northern region of Latin America. TcII predominates in the domestic environment of all South America, presenting a higher resistance to antiparasitic chemotherapy and high pathogenicity (Di Noia *et al.*, 2002; Freitas *et al.*, 2005; Botero *et al.*, 2007). This lineage was initially subdivided into five units of discrete typologies characterized as IIa, IIb, IIc, IId and IIe (Brisse *et al.*, 2000), but

Zingales *et al.* (2009) propound that TcII is no longer divided into five subgroups but each of those subgroups constitutes an independent DTU (TcII–VI). TcIII predominates in the wild environments of South America, with most cases affecting small mammals such as bats and quatis cases being reported in Brazil, more specifically in the Amazon (Lisboa *et al.*, 2009; Rocha *et al.*, 2013), and with only one chronic case found in humans (Abolis *et al.*, 2011). Recent researches agree that TcI and TcII are two pure lineages and that TcV and TcVI have a hybrid origin with TcII and TcIII, while the evolution of TcIII and TcIV still unclear (Zingales, 2018).

Although the relationship between genotype and parasitic phenotype, tropism and clinical manifestations remain poorly understood (Macedo and Pena, 1998; Vago *et al.*, 2000; Prata, 2001), all *T. cruzi* strains isolated from the natural environment have been shown to infect mammalian hosts (Yeo *et al.*, 2005; Herrera, 2010). In vertebrate hosts, *T. cruzi* establishes a systemic infection and parasitism of multiple organs, especially the heart, intestines and oesophagus (Lana and Tafuri, 2016). Although the neurological changes associated with Chagas' disease are often neglected, there is evidence that *T. cruzi* is able to parasite and induce inflammatory lesions in structures of the peripheral nervous system (PNS) (Marin-Neto *et al.*, 2007) and central nervous system (CNS) (Masocha and Kristensson, 2012; Pittella, 2013). The CNS involvement during the acute phase of Chagas' disease can lead to meningitis, seizures, restlessness, continuous crying, insomnia and transient coma (Sangster and Dobson, 2002; Storino *et al.*, 2003). The consequences of chagasic meningoencephalitis that occur at the chronic phase consist of motor and sensory disorders, psychic alterations and cerebellar impairment (Sangster and Dobson, 2002). In addition, electrophysiological changes were determined as a consequence of the deterioration of the cerebral cortical function in individuals with chronic Chagas' disease (Prost *et al.*, 2000).

Currently, PNS alterations are better understood, and dysautonomia secondary to ganglia and nerve endings of the sympathetic and parasympathetic autonomic nervous system have been consistently implicated in the pathophysiology of cardiomyopathy and chagasic megasyndromes (Oliveira *et al.*, 2017). However, tropism, distribution and changes induced by *T. cruzi* in different structures and organs of the CNS are poorly understood. Considering that the current evidence is flawed because it is based on fragmented data, it is difficult to understand the range of the CNS changes that develop throughout the infection with *T. cruzi*. Therefore, from a structured and systematized search, we evaluated the preclinical evidence regarding the impact of *T. cruzi* infection on the CNS. In addition to characterizing the infection models used, we established the relationship between the characteristics of *T. cruzi* strains and their tropism to the CNS and other tissues and organs susceptible to parasitism as well as the most frequent lesions incurred. Moreover, we have critically evaluated the scientific evidence regarding the methodological quality of the studies included in this systematic review.

Materials and methods

Literature search

A comprehensive bibliographic survey completed on 11/20/2017 at 7:30 PM was conducted in the PubMed/Medline databases (https://www.ncbi.nlm.nih.gov/pubmed) and Scopus (https://www.scopus.com/home.uri). Structured descriptors were used in search filters constructed for three domains: Chagas disease, nervous system and animal model (Table S1). The filters on the PubMed/Medline platform were constructed using a hierarchical distribution of the MESH terms. We used the same PubMed search strategy to search the Scopus platform; however, we used the filter for animal studies provided by the Scopus platform. The non-MeSH descriptors were characterized by the algorithm [TIAB], which was also used to retrieve recently published but non-indexed (in-process) studies. This systematic review was developed according to the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analysis; Moher *et al.*, 2009), which is used as a guide for selection, screening and eligibility of studies (Fig. 1).

Data extraction and management

An independent researcher (E.V.) selected eligible studies following the analysis of their titles and abstracts. When in doubt, an arbitration was requested from other independent reviewers (R.V.G, M.M.S. and R.D.N.) to decide whether any given study met the eligibility criteria previously defined, likewise to discard subjectivity in the data collection and selection process, the information was extracted independently and analysed separately. Data from each study were extracted and tabulated using standard information such as: (i) characteristics of the publication (title, author, year and country where the study was performed); (ii) experimental model (animal species, gender, age, weight and the number of animals and of experimental groups); (iii) infection characteristics (nature of infection, *T. cruzi* strain, inoculation route, amount of inoculum and the phase of parasitemia); and (iv) morphological and functional outcomes associated with the CNS (diagnostic test, infected tissue and types of changes). Whenever we encountered difficulties in obtaining the full-text papers, we requested the authors by e-mail to provide a copy of the article. Subsequently, the data were compared and the conflicting information identified and corrected after discussion among the researchers.

Eligibility criteria

Only original studies published in English, Portuguese and Spanish that met the following eligibility criteria were selected: (i) studies with mammals infected experimentally or naturally with *T. cruzi*; (ii) studies with at least one control group infected with *T. cruzi* that was not submitted to any treatment; (iii) studies using naturally occurring and non-genetically engineered strains; (iv) studies with hosts that were not genetically modified and that did not present alterations resulting from other interventions; (v) studies describing CNS-related morphological and/or physiological outcomes; and (vi) full-text studies. Literature reviews, comments, notes, book chapters as well as non-indexed studies were excluded.

Analysis of methodological bias

Bias analysis was structured according to the characteristics described in the ARRIVE strategy (Kilkenny *et al.*, 2010). To this end, we used criteria based on brief descriptions of the essential characteristics of all studies using animal models, such as the theoretical background, research aim, analytical methods, statistical approach, sample calculations and research outcome. A table summarizes all relevant and applicable aspects considering the specificity and the aims of the systematic review. The individual adherence to the bias criteria and the general mean of adhesion are expressed as absolute values (n) and percentage (%) (Pereira *et al.*, 2017).

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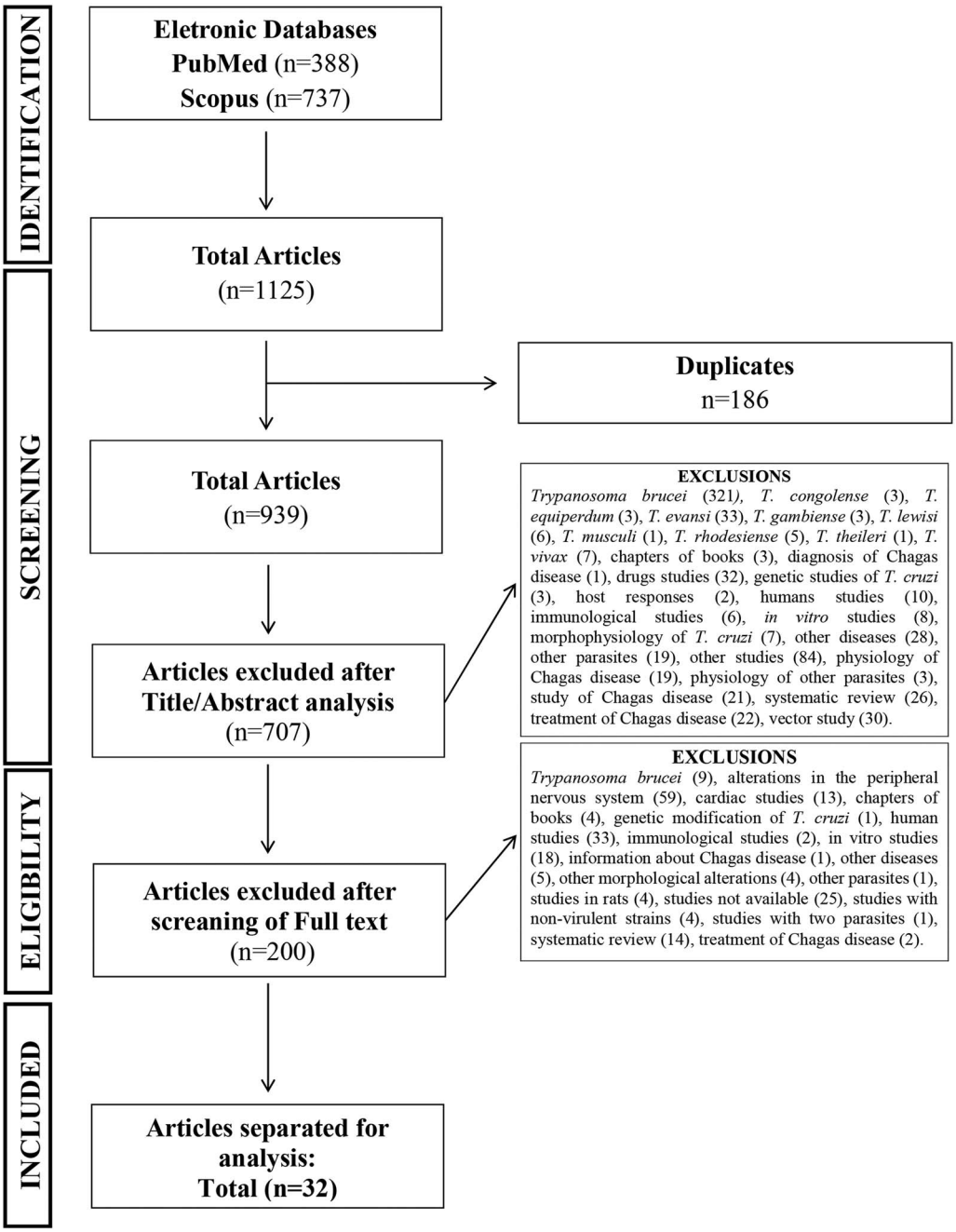

Fig. 1. Flow diagram of search results, study screening and eligibility to define the articles to be included in the systematic review according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyzes; www.prisma-statement.org).

Results

Inclusion of studies

Initial research resulted in 1125 studies, but 186 were excluded because they were duplicate studies. After reading the title and abstract, 707 irrelevant studies were excluded. After the remaining 232 articles were read in their entirety, another 200 articles were excluded including studies describing alterations in the PNS ($n = 59$), clinical studies ($n = 33$), *in vitro* ($n = 18$) and secondary studies ($n = 14$). Finally, 32 studies fully met the inclusion criteria and were included in the systematic review (Fig. 1).

Analysis of infection models

The 32 studies were conducted in seven different countries: Brazil (40.6%; $n = 13$), USA (25%; $n = 8$) and Argentina (12.5%; $n = 4$). The most used animal models were mice (90.6%; $n = 29$), horse, pig and guinea pig (3.1%; $n = 1$ each). The most used mouse lines were C3H (40.6%; $n = 13$), Swiss (25%; $n = 8$) and C57BL/6 (18.8%; $n = 6$). The most used *T. cruzi* isolates were: Colombian (25%; $n = 8$), Brazil (15.6%; $n = 5$), Y, RA and Tulahuén (9.4%; $n = 3$ each). The most frequent route of inoculation was intraperitoneal (68.8%; $n = 22$) followed by subcutaneous, intradermal and intravenous (6.3%; $n = 2$ each). The inoculation route was not reported in four studies (12.5%). Tests to confirm infection were not described in 11 articles (35.4%) (Table 1). Most of the studies evaluated acute infections (62.5%; $n = 20$). Acute and chronic infections were simultaneously reported in eight studies (25%), while exclusively chronic infections were evaluated in only four studies (12.5%) (Table 2).

The most frequently used *T. cruzi* genotypes were: TcI (40.6%; $n = 13$), TcII (12.5%; $n = 4$), TcIV (3.1%; $n = 1$) and TcVI (12.5%;

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Table 1. Characteristics of the studies evaluating the changes in the central nervous system following infection with *T. cruzi*

Reference	Country	Animals	Species	Lineages	Sex	Age (days)	Weight (g)	Amount of animals	Type of infection	*T. cruzi* strains	Route of inoculation	Inoculation (trypomastigotes)	Confirmation of infection
De Diego *et al.* (1991)	ES	Ms	*Mus musculus*	Swiss	?	?	?	85	E	Bolivia	Ip	100 000	?
										Y			
Snary *et al.* (1983)	GB	Ms	*Mus musculus*	BALB/c	?	?	?	?	E	Esmeraldo cl3	?	?	?
Nisimura *et al.* (2014)	BR	Ms	*Mus musculus*	Swiss Webster	♂	42–48	18–20	20	E	Y	Ip	10 000	FBE
Tanowitz *et al.* (1983)	US	Ms	*Mus musculus*	A/J	♀	49	?	?	E	Brasil	Ip	10 000	Para
				C3H									
Monteiro *et al.* (2012)	BR	Ms	*Mus musculus*	Swiss	♂	12–15	?	49	E	AM49	Ip	1 000 000	FBE HC
Castro-Sesquen *et al.* (2011)	PE	Gp	*Cavia porcellus*	Andean	♀	60	600–700	90	E	Y	Id	10 000	MHCT ELISA
Silva *et al.* (1999*a*)	BR	Ms	*Mus musculus*	C3H/He	♀	35–49	?	12	E	Colombiana	Ip	100	FBE
Bryan *et al.* (2016)	US	Hs	*Equus ferus*	Quarter horse	♂	3650	?	1	N	?	?	?	PCR
Tekiel *et al.* (1997)	AR	Ms	*Mus musculus*	C3H/HeN	♂	420	?	64	E	RA	Ip	10–50	MHCT
										CA-I		100 000	
Tekiel *et al.* (2005)	AR	Ms	*Mus musculus*	C3H/HeN	♂	420	?	16	E	RA	Idp	10–30	FBE ELISA
Meza *et al.* (2014)	BR	Ms	*Mus musculus*	Swiss	♂	21–28	?	110	E	AM05	Ip	10 000	FBE
										AM18			
										AM62			
										AM64			
										AM67			
										AM68			
										PR1226			
										PR2259			
Hanson and Roberson. (1974)	US	Ms	*Mus musculus*	Albino CF1	♀	28–70	?	125	E	Brasil	Ip	50 000	FBE XD
Buckner *et al.* (1999)	US	Ms	*Mus musculus*	C3H/He	♀	42–56	?	45	E	Tulahuen	Sc	250	FBE
Yauri *et al.* (2016)	PE	Pg	*Sus scrofa* domestica	Cross-bread	♀	60	?	5	E	Boliviana	Iv	1 000 000/kg	FBE
											Id	1 000 000/kg	
											Iv	5 000 000/kg	

											Id	5 000 000/kg	
Roffê *et al*. (2003)	BR	Ms	*Mus musculus*	C3H/He C57BL/6	♀	35–49	?	32	E	Colombiana	Ip	100	FBE
Mirkin *et al*. (1994)	AR	Ms	*Mus musculus*	C3H/HeN	?	28	?	?	E	CA-I RA	Ip	100 000 50–100	MHCT FBE
Silva *et al*. (1999*b*)	BR	Ms	*Mus musculus*	C3H/He	♀	35–49	?	30	E	Colombiana	Ip	100	FBE
Bombeiro *et al*. (2012)	BR	Ms	*Mus musculus*	C57BL/6	♀	56–70	?	42	E	Sylvio X10/4	Ip	100 000	?
Andrade *et al*. (1997)	BR	Ms	*Mus musculus*	Swiss	?	?	15–18	100	E	Colombia Bolivia Montalvania	?	4000	?
Guarner *et al*. (2001)	US	Ms	*Mus musculus*	DBA/2	♂	42–56	?	?	E	Brasil	Ip	20 000	?
Michailowsky *et al*. (2001)	BR	Ms	*Mus musculus*	BALB/c C57BL/6	♀	42–56	?	?	E	Colombiana	Ip	5000	FBE
Caradonna and PereiraPerrin (2009)	US	Ms	*Mus musculus*	C57BL/6 BALB/c	♀	42–56	?	?	E	Tulahuén	Sc In Sc In	5000 25 000 5000 25 000	PCR
De Diego *et al*. (1998)	ES	Ms	*Mus musculus*	Swiss	♂	?	?	24	E	Genotype 19§ Genotype 20 II Genotype 39#	Ip	106	?
de Queiroz and Castro Filho (1985)	BR	Ms	*Mus musculus*	Swiss	?	?	?	?	E	Colombiana	Ip	100 000	?
Kuhn *et al*. (1974)	US	Ms	*Mus musculus*	C3H/He	♀	?	18–20	?	E	Brasil	Iv	10 000 000	?
Molina *et al*. (1987)	AR	Ms	*Mus musculus*	C3H/HeN	♂	630	?	24	E	Tulahuén	Ip	50	S
Tanowitz *et al*. (1981)	US	Ms	*Mus musculus*	C3H/HeJ	♀	42–68	?	?	E	Brasil	Ip	10 000	?
Morocoima *et al*. (2012)	VE	Ms	*Mus musculus*	NMRI Albino	?	20	12	50	E	TRPX/VE/2009/RP3 TTMA/VE/2009/TMG1 MDID/VE/2009/RC1 MDID/VE/2009/AM10	Ip	4000	FBE

(*Continued*)

Table 1. (*Continued.*)

Reference	Country	Animals	Species	Lineages	Sex	Age (days)	Weight (g)	Amount of animals	Type of infection	*T. cruzi* strains	Route of inoculation	Inoculation (trypomastigotes)	Confirmation of infection
Andrade *et al.* (2002)	BR	Ms	*Mus musculus*	BALB/c	♂	?	?	?	E	Col1.7G2	Ip	50	PCR
				DBA/2						Col1.7G2			
				Swiss						JG			
Vilar-Pereira *et al.* (2012)	BR	Ms	*Mus musculus*	C3H/He (H-2k)	♀	28–42	15–22	?	E	Colombiana	Ip	100	FBE
				C57BL/6 (H-2b)									
Silva *et al.* (2007)	BR	Ms	*Mus musculus*	C3H/HeJ (H-2k)	♀	35–49	?	?	E	Colombiana	Ip	100	?
De Scorza *et al.* (1989)	VE	Ms	*Mus musculus*	NMRI	?	?	5 10 15	36	E	VP1	?	?	?
										VP2			
										VP5			
										VP7			

AR, Argentina; BR, Brazil; ES, Spain; GB, United Kingdom; PE, Peru; US, United States; VE, Venezuela; Ms, mouse; Hs, horse; Gp, guinea pig; Pg, pig; ♂, male; ♀, female; ?, uninformed; E, experimental; N, natural; §, strains OPS21, SP104, 13379, Gamba; II, strains P11, ESQUILO, CUICA, P209, SO34; #, strains SO3, NR, BUG2148, BUG2149, MN, SC43; Ip, intraperitoneal; Id, intradermal; Idp, intradermoplantar; Sc, subcutaneous; Iv, intravenous; In, intranasal; FBE, fresh blood examination; HC, haemoculture; MHCT, microhaematocrit centrifugue technique; PCR, polymerase chain reaction; ELISA, Enzyme-Linked ImmunoSorbent Assay; Para, parasitaemia; XD, xenodiagnostic; S, serology.

$n = 4$). Some studies used more than one genotype (18.8%; $n = 6$); however, four studies (12.5%) did not identify the genotype of the strains. Histopathological analyses were performed in 23 studies (71.9%), six studies used immunohistochemistry (18.8%), six used polymerase chain reaction (18.8%), and three did Western-blot analysis (9.4%). The CNS organs with the largest changes were brain (65.6%, $n = 21$), followed by the spinal cord (25%; $n = 8$) and cerebellum (15.6%; $n = 5$) (Fig. 2).

The most frequent lesions in the CNS were the presence of inflammatory foci (68.8%; $n = 22$), with a predominance of lymphocytic mononuclear infiltrate (15.6%; $n = 5$). The encephalon presented moderate-to-intense inflammation with a marked perivascular distribution. To a lesser extent, inflammatory foci were found in the meninges (9.4%, $n = 3$), choroid plexus (9.4%, $n = 3$) and nuclei at the base (6.3%; $n = 2$). In the spinal cord, inflammatory foci were found mainly associated with nerve roots (50%, $n = 16$) and meninges (50%, $n = 16$) (Table 2; Fig. 2).

The presence of amastigote nests, free trypomastigotes or indeterminate forms of *T. cruzi* in the CNS was reported in 53.1% of the studies ($n = 17$). The presence of amastigotes in the cytoplasm of glial cells (astrocytes, microglia, ependymocytes and oligodendrocytes) was observed in the organs or tissues with the highest presence of parasites (68.8%; $n = 22$). Pseudocysts with intra and extracellular amastigotes were also found in the nuclei of the base (12.5%, $n = 4$), cerebellum (12.5%, $n = 4$) and Purkinje cells (12.5%; $n = 4$). Amastigotes were found in the white matter of the spinal cord, intra and extracellular (9.4%; $n = 3$).

The presence of anti-*T. cruzi* antibodies was described in three studies (9.4%) and *T. cruzi* antigens in four studies (12.5%). Vasculopathies were reported in five studies (15.6%), gliosis in three (9.4%), satellitosis in two (6.3%), while tissue damage due to necrosis and oedema was described in the cerebrum and spinal cord in three studies each (9.4%) (Table 2, Fig. 2). Only 11 studies (34.4%) evaluated the parasitic load on the day the animals were sacrificed, ranging from 0 to 69.3×10^6 trypomastigotes.

The rare reports covering the chronic phase of Chagas' disease indicated inflammatory foci ranging from light to intense (9.4%; $n = 3$), presence of *T. cruzi* nests (6.3%; $n = 2$), tissue damage as a result of autoimmune lesions (3.1%, $n = 1$), and neuron degeneration and necrosis (3.1%, $n = 1$) were the most frequent alterations. The most affected sites were the brain, the blood–brain barrier (BBB) and the spinal cord.

Bias analyses

The results regarding the bias analyses are shown in Table 3. An average of 55.0 ± 12.3 ARRIVE items were met by the original studies. In general, studies performed up to 15 years ago were those that presented the greatest deficiency in the methodological detail and the description of the results (Fig. 3). Only seven articles (21.9%) justified the animal model used. Approval of the ethics committee was reported in 13 studies (40.6%). Only two studies (6.25%) justified the size of the *T. cruzi* inoculum used. No study justified the route of administration. All studies ($n = 32$) indicated the animal species and the *T. cruzi* strain used. The sex, weight and age of the animals were described in 84.4% ($n = 27$), 25% ($n = 8$) and 75% ($n = 24$) of the studies, respectively. Calculation of the sample size was made explicit in only one study (3.1%). The detailed description of the statistical analyses used was reported in 43.8% of the studies ($n = 14$). Sixteen studies (50%) reported modifications to the experimental protocol by adverse events (Table 3).

Discussion

Using a systematic screening, we observed that most of the studies investigating CNS changes caused by *T. cruzi* were conducted in

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Table 2. Changes in CNS tissues or organs during *T. cruzi* infection

Reference	Strain of *T. cruzi*	Genotypes of *T. cruzi* +	Geographical origin ++	Change assessment	Central nervous system		Phase	Parasitic burdens 106/mL
					Organ/tissue/tropism	Alterations		
Hanson and Roberson (1974)	Brazil	TcI	American continent	Histpat	Telencephalon	Presence of amastigotes/pseudocysts	Acute	3.6
					Cerebellum			
Kuhn *et al*. (1974)	Brazil	TcI		Radlab	Brain	Presence of *T. cruzi* in the first 30 h	Acute	?
Tanowitz *et al*. (1981)	Brazil	TcI		EA	Brain	Decreased choline acetyltransferase	Chronic	18
Tanowitz *et al*. (1983)	Brazil	TcI		Bαb	Brain	↑ Nicotinic receptors of ACh Neuronal denervation	Acute	?
de Queiroz and Filho (1985)	Colombian	TcI		Histpat	Choroid plexus	Small inflammatory foci or isolates with predominance of lymphocytes	Acute	?
					Meninges	Presence of inflammatory infiltrates		
Silva *et al*. (1999*a*)	Colombian	TcI		Histpat Imnhisq	Brain parenchymal	Intense inflammatory infiltrates*	Acute chronic**	69.3
					Meninges	Inflammatory infiltrates* of mild–moderate		
					Choroid plexus	Intense inflammatory infiltrates*		
					Hippocampus	Intense inflammatory infiltrates*		
					Perivascular space	Oedema		
						Increase in size		
						Intense inflammatory infiltrates*		
					Cerebellum	Intense inflammatory infiltrates*		
					Blood–brain barrier	Random inflammatory* foci		
Silva *et al*. (1999*b*)	Colombian	TcI	American continent	Histpat Imnhisq	Encephalon	Presence of *T. cruzi* antigens	Acute	?
						Intense inflammatory infiltrates in meninges, leptomeninges, choroid plexus and basal lamina of BV		
						Incomplete areas of the BBB		
						Moderate presence of extracellular matrix antibodies FN, α4, LN, α5, α6		
						Inflammatory infiltrates very few	Chronic	
						Moderate presence of extracellular matrix antibodies FN, α4, LN, α5, α6		

(*Continued*)

Table 2. (*Continued.*)

Reference	Strain of *T. cruzi*	Genotypes of *T. cruzi* +	Geographical origin ++	Change assessment	Central nervous system		Phase	Parasitic burdens 106/mL
					Organ/tissue/tropism	Alterations		
Guarner *et al.* (2001)	Brazil	TcI	American continent	Histpat Imnhisq	Encephalon	Intense presence of amastigotes in astrocytes and ependymocytes, and few in oligodendrocytes	Acute	?
						Presence of *T. cruzi* antigens in connective tissue surrounding		
						Moderate focal perivascular inflammation		
						Rare presence of amastigotes in astrocytes	Chronic	
						Vacuolar degeneration		
						Mild focal perivascular inflammation		
Michailowsky *et al.* (2001)	Colombian	TcI		Histpat	Brain	Rare nests of amastigotes isolated	Acute	0.00145
						Inflammatory infiltrates		
Roffê *et al.* (2003)	Colombian	TcI		Histpat Imnhisq	Encephalon	Focal Meningoencephalitis	Acute	?
						Intense perivascular and parenchymal mononuclear†† infiltrates irregularly distributed		
						Presence of *T. cruzi* antigens		
						Mild mononuclear†† infiltrates restricted to areas of incomplete BBB	Chronic	
						Moderate mononuclear†† infiltrates restricted to areas of incomplete BBB	Acute	
						Presence of *T. cruzi* antigens		
						Mild mononuclear†† infiltrates restricted to areas of incomplete BBB	Chronic	
Silva *et al.* (2007)	Colombian	TcI	American continent	Imnhisq	Brain	Presence of lymphocytic inflammatory infiltrates T CD8+	Acute	?
						Meningoencephalitis		
Bombeiro *et al.* (2012)	Sylvio X10/4	TcI		Histpat Imnhisq PCR	Spinal cord	Astrogliosis in white and grey matter	Acute	?
						Increased density of macrophages and microglia		
						Rare inflammatory foci		
						Presence of inflammatory molecules CD3, TNF-α, IFN-y, iNOS, IL-10		
						Presence of *T. cruzi*		

Vilar-Pereira *et al.* (2012)	Colombian	TcI		Histpat RT-PCR	Brain	Presence of amastigotes in astrocytes, microglia, hippocampus and cerebral parenchyma	Acute	0.9
						Inflammatory infiltrates in the cerebral parenchyma, perivascular spaces and hippocampus		
						Presence of amastigotes in astrocytes, microglia and cerebral parenchyma		
Snary *et al.* (1983)	Esmeraldo cl3	TcII	South America	Dot Blot IFAT Western blot	Brain Spinal cord	Presence of monoclonal antibodies (5H7 and CE5)	Acute	?
De Diego *et al.* (1991)	Bolivia	TcII		Histpat	Brain	Inflammation	Acute	?
	Y					Inflammation Pseudocyst		
Castro-Sesquen *et al.* (2011)	Y	TcII		Histpat PCR	Brain	Presence of amastigotes Tissue damage	Acute chronic	0.0059
Nisimura *et al.* (2014)	Y	TcII		TBARS	Brain	↑ Oxidative stress	Acute	39.8
				DAAch		Microvasculopathy		
Monteiro *et al.* (2012)	AM49	TcIV	North and South America	Histpat	Brain	Mild inflammatory foci Gliosis	Acute	0.0014
Molina *et al.* (1987)	Tulahuén	TcVI	?	Histpat	Spinal cord	Mild inflammatory¶ infiltrates in the ventral nerve root	Chronic	?
						Moderate inflammatory¶ infiltrates in the meninges		
						Intense inflammatory¶ infiltrates in the spinal tissue		
						Presence of *T. cruzi* nests		
						Degeneration and necrosis of neurons***		
						Increased microglial proliferation		
Buckner *et al.* (1999)	Tulahuén	TcVI		Histpat	Brain	Presence of amastigotes	Acute	0.0006755
						Mild inflammatory cell infiltrates		
Tekiel *et al.* (2005)	RA	TcVI		PCR	Spinal cord	Inflammatory infiltrates (lymphocytes T CD4+ and CD8+)	Chronic	?
						Autoimmune lesions		
Caradonna and PereiraPerrin (2009)	Tulahuén	TcVI		Histpat PCR	Cerebral cortex	Moderate presence of *T. cruzi*	Acute	0.054
					Basal nuclei	Intense presence of *T. cruzi*		
						Few inflammatory foci		
					Cerebellum	Rare presence of *T. cruzi*		
					Cerebral cortex	Moderate presence of *T. cruzi*		0

(*Continued*)

Table 2. (*Continued.*)

Reference	Strain of *T. cruzi*	Genotypes of *T. cruzi* +	Geographical origin ++	Change assessment	Central nervous system		Phase	Parasitic burdens 106/mL
					Organ/tissue/tropism	Alterations		
					Basal nuclei	Intense presence of *T. cruzi*		
						Abundant inflammatory foci		
					Cerebellum	Rare presence of *T. cruzi*		
					Brain	Moderate presence of *T. cruzi*		0
						Intense presence of *T. cruzi*		0.020
Mirkin *et al.* (1994)	CA-I	TcI		Histpat Imnhisq	Spinal cord	Mild vasculitis	Acute	?
			American continent			Mild meningeal lymphomononuclear† infiltrates		
						Satellitosis		
						Chronic leptomeningitis	Chronic	
	RA	TcVI	?			Mild inflammatory† infiltrates were limited to dorsal and ventral roots and to dorsal root ganglia	Acute	
							chronic	
Andrade *et al.* (1997)	Colombia	?	?	Histpat	Meninges	Focal perivascular mononuclear infiltration	Acute	?
	Bolivia	TcII	American continent					
	Montalvania	TcI			Choroid plexus	Diffuse mononuclear infiltrate		
Tekiel *et al.* (1997)	RA	TcVI	?	Western blot	Brain	Presence of three *T. cruzi* antigens	Acute chronic	?
	CA-I	TcI	American continent		Spinal cord			
De Diego *et al.* (1998)	Genotype 19§	?	?	Histpat	Brain	Few inflammatory foci‡	Acute	?
	Genotype 20II	?	?			Few inflammatory foci with greater amount of cells‡		
						Inflammatory foci around the BV of the leptomeninges		
						Liquefactive necrosis		
						Presence of amastigote nests		
	Genotype 39#	TcV	?			Few inflammatory foci‡		
Andrade *et al.* (2002)	Col1.7G2	TcI	American continent	PCR LSSP-PCR	Brain	Presence of *T. cruzi*	Chronic	?
						Inflammatory foci		

	Col1.7G2	TcI				Presence of *T. cruzi*		
	JG	TcII	South America			Inflammatory foci		
Meza *et al.* (2014)	AM05	TcIV	North and South America	Histpat	Brain	Mild inflammation	Acute	0
	AM18					Mild inflammation		
	AM62					Presence of amastigote nests		
	AM64					Mild inflammation		
	AM67					Mild and focal inflammation		
						Gliosis		
	AM68					Mild inflammation		
	PR1226	TcII	South America			Mild-to-moderate inflammation		
	PR2259					Moderate and focal inflammation		
De Scorza *et al.* (1989)	VP1	?	?	Histpat	Brain	Few nests of amastigotes in the microglia	Acute	?
						Few nests of amastigotes in the white matter		
	VP2					Few nests of amastigotes in the white matter		
						Discrete inflammatory foci		
	VP5					Few nests of amastigotes in the white matter		
	VP7					Few nests of amastigotes in the white matter		
					Cerebellum	Presence of amastigote nests		
Morocoima *et al.* (2012)	TRPX/VE/2009/RP3 TTMA/VE/2009/TMG1 MDID/VE/2009/RC1(b)	?	?	Histpat	Brain	Presence of amastigotes/trypomastigotes in astrocyte cytoplasm	Acute	?
						Presence of amastigotes/trypomastigotes on neuropil of white matter		
					Cerebellar cortex	Presence of amastigote nests in astrocytes		
						Neuropilic oedema		
					Cerebellum granular layer	Presence of amastigotes/trypomastigotes in microglia		
						Neuropilic oedema		
						Satellitosis		
					Cerebellar leptomeninges	Presence of amastigotes		
					Purkinje layer cells	Presence of amastigote nests in astrocytes		

(*Continued*)

Table 2. (*Continued.*)

Reference	Strain of *T. cruzi*	Genotypes of *T. cruzi* +	Geographical origin ++	Change assessment	Central nervous system		Phase	Parasitic burdens 106/mL
					Organ/tissue/tropism	Alterations		
					Cerebellar white matter			
					Spinal cord	Presence of intra and extracellular pseudocysts		
					Basal nuclei			
	MDID/VE/2009/AM10				Brain	Presence of amastigotes/trypomastigotes in astrocyte cytoplasm		
						Presence of amastigotes/trypomastigotes on neuropil of white matter		
Bryan *et al.* (2016)	?	?	?	Histpat	Spinal cord	Inflammatory infiltrates (lymphocytes, plasma cells and macrophages) in the meninges, white and grey matter	?	?
						Axons mildly swollen and demyelinated		
						Pseudocysts on white matter		
						Necropsy		
Yauri *et al.* (2016)	Bolivian	?	?	Histpat Western blot PCR	Brain	Presence of amastigotes	Acute	?
						Mild-to-moderate perivasculitis		
						Presence of antibodies IgG anti-*T. cruzi*		
						Mild to moderate perivasculitis (lymphocytes)		
						Presence of antibodies IgG anti-*T. cruzi*		

+, Volpato *et al.*, 2017; Meza *et al.*, 2014; Alves *et al.*, 2012; Minning *et al.*, 2011; Andrade *et al.*, 2010; Zingales *et al.*, 2009; ++, Zingales *et al.*, 2012; ?, uninformed; ↑, increase; *, (macrophages, CD8+ and CD4+); **, during the chronic phase inflammatory infiltrates were mild or non-existent; ††, macrophages (data not shown) and CD8+ and, to a lesser extent, CD4+ T cells; †, CD8+ predominant respect to CD4 +; ‡, prevalence of macrophages, mononuclear cells and microglia; §, strains OPS21, SP104, 13379, Gamba; II, strains P11, ESQUILO, CUICA, P209, SO34; #, strains SO3, NR, BUG2148, BUG2149, MN, SC43; ¶, composed of lymphocytes, macrophages and occasional polymorphonuclear cells; ***, the greater inflammatory foci, the smaller the decrease in the number of neurons; ACh, acetylcholine; (b), without alterations in the brain; LSSP-PCR, low-stringency single specific primer; PCR, polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; Histpat, histopathological; Imnhisq, immunohistochemistry; DAACh, dilation of cerebral arterioles with acetylcholine; Bαb, binding of α-bungarotoxin; IFAT, immunofluorescence antibody test; TBARS, thiobarbituric acid reactive substances species; EA, enzyme assay; Radlab, radiolabelled; BBB, blood–brain barrier; FN, fibronectina; LN, laminin; BV, blood vessels.

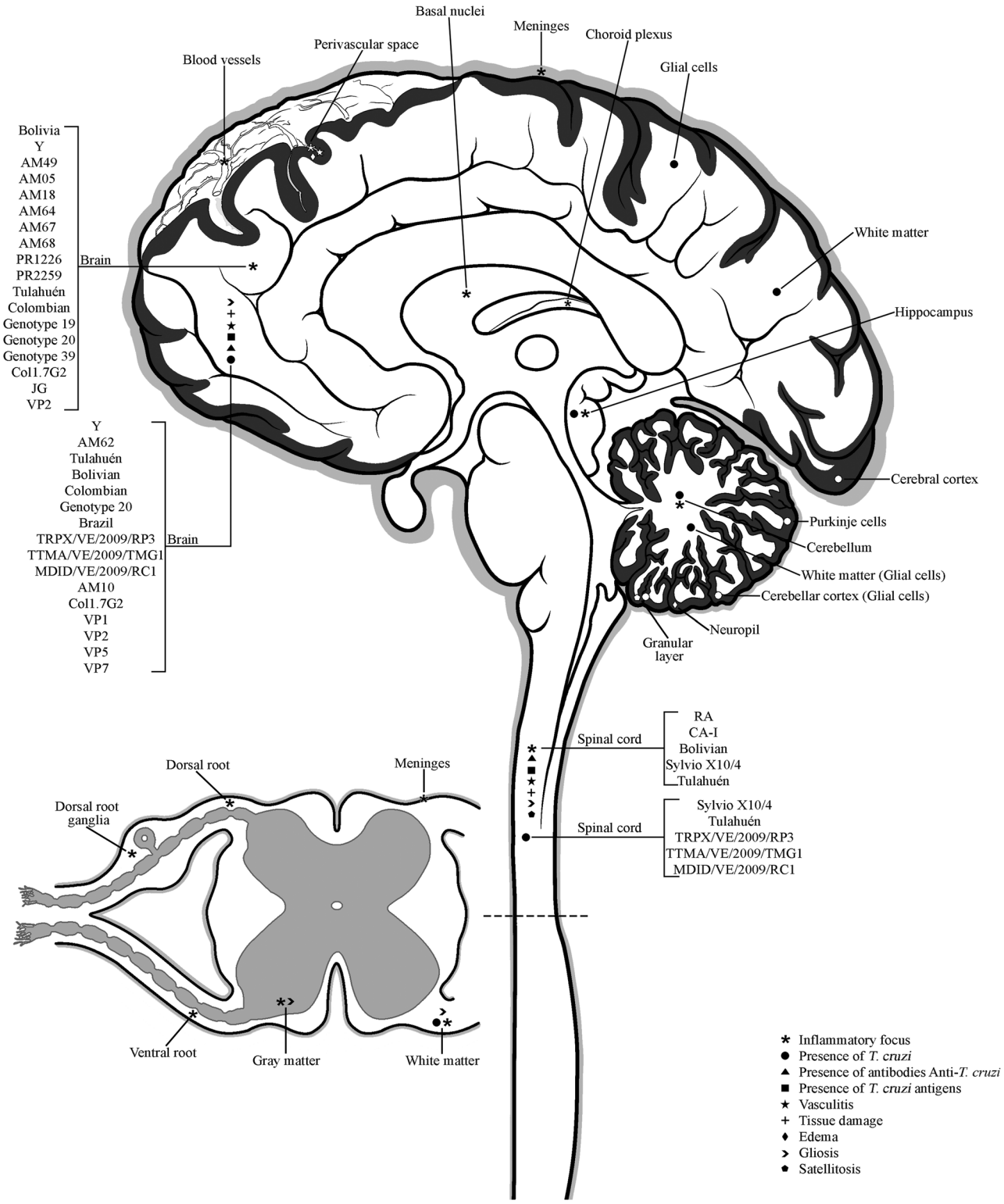

Fig. 2. Schematic representation demonstrating the distribution of morphological changes and tropism of the different strains of *T. cruzi* in the CNS. *: inflammatory focus; ●: presence of *T. cruzi*; ▲: presence of anti-*T. cruzi* antibodies; ■: presence of *T. cruzi* antigens; ★: vasculopathy; +: tissue damage; ♦: oedema; : gliosis; : satellitosis. The predominant strains in each region are presented in square brackets [.

developing countries, corroborating the idea that research efforts about this parasite are concentrated in countries where Chagas' disease is endemic (Antinori *et al.*, 2017). In addition, the overall methodological quality score for this set of studies was limited. Since the bias analysis presented herein was structured following the basic requirements for the rational acquisition and interpretation of results, the limited quality of the evidence can be attributed to studies with low individual methodological scores (Zoltowski *et al.*, 2014). These aspects point to an urgent need for more rigorous analysis and interpretation of the evidence considering all the critical elements that may undermine the validity of the studies. Interestingly, our results also showed a temporal influence on the bias variation because older studies presented poor descriptions of the experiments and only met a few criteria established by the bias analysis. Nevertheless, our findings show that there has been an improvement in the detail presented by

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Table 3. Bias analysis (ARRIVE) of studies with changes in the central nervous system during infection with *T. cruzi*

Quality criteria	Kuhn *et al.* (1974)	Hanson and Roberson. (1974)	Tanowitz *et al.* (1981)	Tanowitz *et al.* (1983)	Snary *et al.* (1983)	de Queiroz e Filho (1985)	Molina *et al.* (1987)	De Scorza *et al.* (1989)	De Diego *et al.* (1991)	Mirkin *et al.* (1994)	Andrade *et al.* (1997)	Tekiel *et al.* (1997)	De Diego *et al.* (1998)	Silva *et al.* (1999*b*)	Silva *et al.* (1999*a*)	Buckner *et al.* (1999)	Michailowsky *et al.* (2001)	Guarner *et al.* (2001)	Andrade *et al.* (2002)	Roffé *et al.* (2003)	Tekiel *et al.* (2005)	Silva *et al.* (2007)	Caradonna and PereiraPerrin (2009)	Castro-Sesquen *et al.* (2011)	Vilar-Pereira *et al.* (2012)	Morocoima *et al.* (2012)	Bombeiro *et al.* (2012)	Monteiro *et al.* (2012)	Meza *et al.* (2014)	Nisimura *et al.* (2014)	Yauri *et al.* (2016)	Bryan *et al.* (2016)	Total (%)
TITLE																																	
Accurate and concise description of the content of the article	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100.00
ABSTRACT																																	
Summary of the background, objectives, methods, main findings and conclusions		✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	93.75
INTRODUCTION																																	
a. Sufficient scientific background		✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	93.75
b. Rational explanation of the experimental approach			✓															✓		✓				✓			✓				✓	✓	21.87
OBJECTIVES																																	
Clear primary and secondary objectives	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	93.75
METHODS																																	
Ethical statement																																	
Ethical permissions									✓								✓				✓		✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	40.63
Study design																																	
a. Number of animals used in the experiment		✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	87.50

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b The experiment was performed as a blind controlled study		✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	87.50
Experimental procedures																																
Treatment dosage	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	93.75
Site and route of administration	✓	✓	✓	✓			✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	90.63
Duration of treatment	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	96.88
Time of day for treatment administration																																0.00
Rational analysis for selection of the specific dosage										✓																				✓		6.25
Rational analysis for specific route of inoculation																																0.00
Experimental animals																																
Information about animals species	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100.00
Animals strains	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	96.88
Animals sex	✓	✓	✓	✓		✓	✓			✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	84.88
Animals body weight	✓					✓		✓			✓														✓	✓	✓				✓			25.00
Animals age		✓	✓	✓			✓	✓		✓		✓		✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	75.00
Description of genetics modifications status (knock-out, transgenic, SPF)				✓																							✓						6.25
Information related to previous procedures performed on animals																								✓						✓			6.25
Housing and husbandry																																
Housing of experimental animals (type of facility, type of housing)							✓							✓			✓		✓	✓			✓		✓	✓		✓	✓	✓			34.38

(*Continued*)

Table 3. (*Continued.*)

Quality criteria	Kuhn *et al.* (1974)	Hanson and Roberson. (1974)	Tanowitz *et al.* (1981)	Tanowitz *et al.* (1983)	Snary *et al.* (1983)	de Queiroz e Filho (1985)	Molina *et al.* (1987)	De Scorza *et al.* (1989)	De Diego *et al.* (1991)	Mirkin *et al.* (1994)	Andrade *et al.* (1997)	Tekiel *et al.* (1997)	De Diego *et al.* (1998)	Silva *et al.* (1999*b*)	Silva *et al.* (1999*a*)	Buckner *et al.* (1999)	Michailowsky *et al.* (2001)	Guarner *et al.* (2001)	Andrade *et al.* (2002)	Roffé *et al.* (2003)	Tekiel *et al.* (2005)	Silva *et al.* (2007)	Caradonna and PereiraPerrin (2009)	Castro-Sesquen *et al.* (2011)	Vilar-Pereira *et al.* (2012)	Morocoima *et al.* (2012)	Bombeiro *et al.* (2012)	Monteiro *et al.* (2012)	Meza *et al.* (2014)	Nisimura *et al.* (2014)	Yauri *et al.* (2016)	Bryan *et al.* (2016)	Total (%)
Husbandry conditions (breeding programme, light/dark cycle, temperature, quality of water)							✓			✓					✓			✓		✓	✓		✓		✓	✓		✓	✓	✓			37.50
Sample size																																	
Number of animals used in each experiment and in each experimental group		✓					✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓			✓		✓	✓	✓	✓	✓	✓	✓	✓	71.88
Explanation regarding number of animals and details of sample size calculation																																✓	3.13
Indicate the number of independent replicates of each experiment, if relevant.				✓	✓				✓			✓			✓									✓					✓		✓	✓	✓	31.25
Distribution of animals in experimental groups																																	
Details of animals allocation to experimental groups (randomization or matching)		✓					✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓			✓		✓	✓	✓	✓	✓	✓	✓		9.38
Treatment strategy: order in which the animals were treated and infected		✓					✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓			✓		✓	✓	✓	✓	✓	✓	✓		9.38

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Experimental outcomes																																
Clear experimental outcomes assessed	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100.00
Statistical methods																																
Statistical methods used for analysis		✓						✓	✓	✓					✓			✓			✓	✓	✓	✓		✓	✓	✓	✓				43.75
Specification of the unit of analysis for each dataset								✓	✓	✓	✓		✓		✓	✓		✓	✓	✓	✓		✓			✓		✓	✓	✓	✓			53.13
Describe the methods used in the statistical approach																												✓					3.13
RESULTS																																
Baseline data																																
Relevant characteristics and health status of animals																		✓										✓		✓	✓		12.50
Numbers analysed																																
Number of animals in each group included in each analysis	✓	✓		✓	✓		✓	✓	✓	✓	✓			✓	✓		✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	✓	✓			71.88
Animals or data not included in the analysis		✓					✓			✓	✓		✓					✓			✓				✓	✓	✓	✓	✓			37.50
Outcomes and estimation																																
Report the results for each analysis carried out (mean±SD)	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	90.63
Adverse events																																
a. Give details of all important adverse events in each experimental groups	✓		✓	✓				✓	✓		✓			✓	✓				✓	✓			✓				✓	✓	✓	✓	✓		50.00
b. Describe any modifications to the experimental protocols made	✓		✓	✓				✓	✓		✓			✓	✓				✓	✓			✓				✓	✓	✓	✓	✓		50.00

(*Continued*)

Table 3. (*Continued.*)

Quality criteria	Kuhn *et al.* (1974)	Hanson and Roberson. (1974)	Tanowitz *et al.* (1981)	Tanowitz *et al.* (1983)	Snary *et al.* (1983)	de Queiroz e Filho (1985)	Molina *et al.* (1987)	De Scorza *et al.* (1989)	De Diego *et al.* (1991)	Mirkin *et al.* (1994)	Andrade *et al.* (1997)	Tekiel *et al.* (1997)	De Diego *et al.* (1998)	Silva *et al.* (1999*b*)	Silva *et al.* (1999*a*)	Buckner *et al.* (1999)	Michailowsky *et al.* (2001)	Guarner *et al.* (2001)	Andrade *et al.* (2002)	Roffé *et al.* (2003)	Tekiel *et al.* (2005)	Silva *et al.* (2007)	Caradonna and PereiraPerrin (2009)	Castro-Sesquen *et al.* (2011)	Vilar-Pereira *et al.* (2012)	Morocoima *et al.* (2012)	Bombeiro *et al.* (2012)	Monteiro *et al.* (2012)	Meza *et al.* (2014)	Nisimura *et al.* (2014)	Yauri *et al.* (2016)	Bryan *et al.* (2016)	Total (%)
to reduce adverse events																																	
DISCUSSION																																	
Interpretation/ scientific implications																																	
a. Interpretation of the results, taking into account objectives, hypotheses, current theory and relevant studies	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100.00
b. Comments on the study limitations (bias, limitations of model)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100.00
c. Describe any findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research																																	0.00
Generalizability/ translation																																	
Comments on how the findings are likely to translate to other species or relevance to human biology	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100.00
Funding																																	
List of funding sources	✓		✓			✓	✓			✓		✓	✓		✓	✓				✓	✓	✓	✓		✓	✓		✓	✓		✓		56.25
Total results (numbers)	15	21	21	24	14	12	21	19	25	26	20	28	22	18	24	23	25	20	18	30	25	25	24	30	24	21	27	28	28	34	32	33	

Unmarked cells indicate that the criteria were not filled

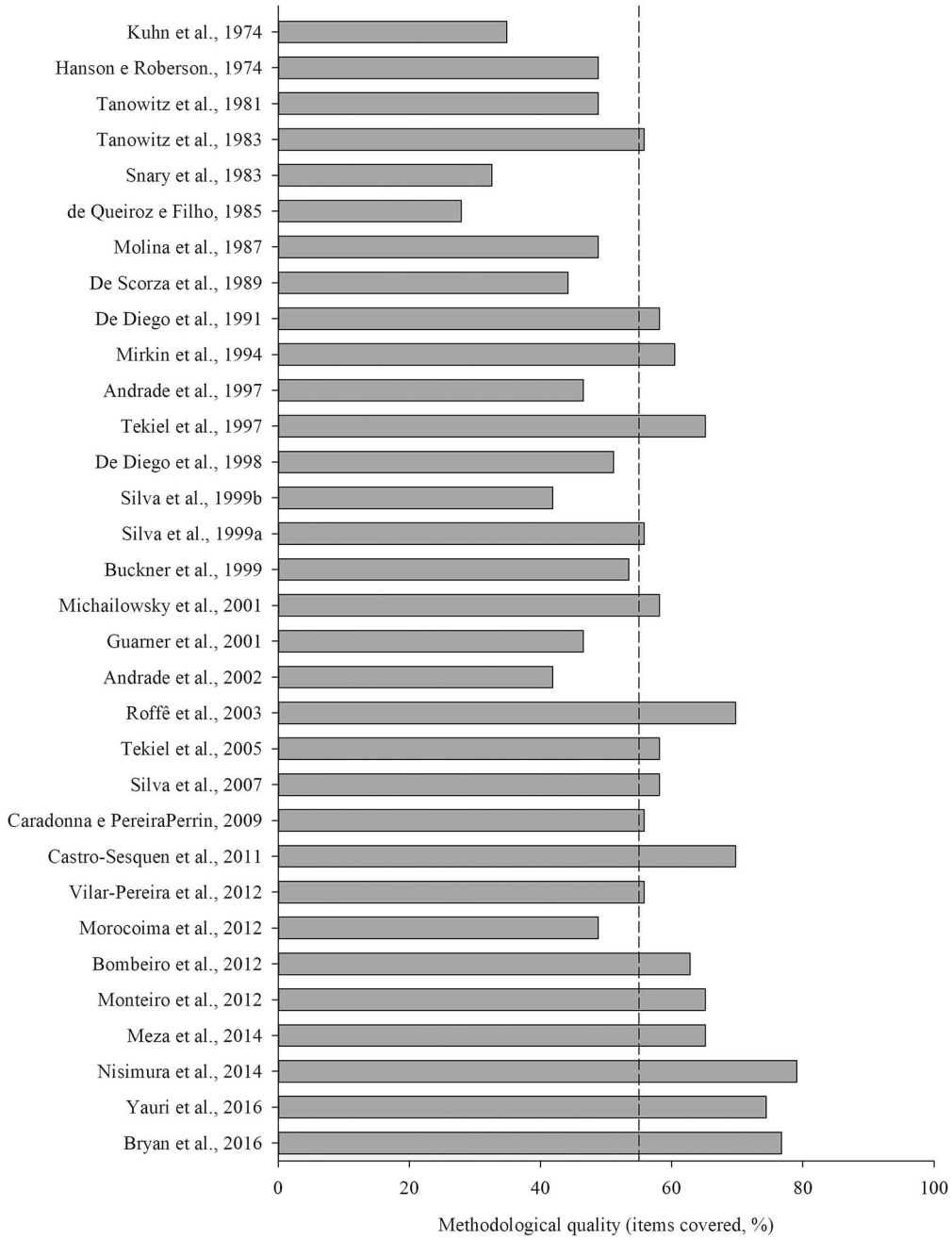

Fig. 3. Analysis of the methodological bias (quality of the report) for each study included in the systematic review according to the ARRIVE guidelines (www.nc3rs.org.uk/arrive-guidelines). The dotted line indicates the average quality score (%). The detailed bias analysis, stratified by domains and evaluated items, is presented in Table 3.

the studies over the years, probably due to the development of new techniques and statistical methods as well as the increase in the availability of guidelines and regulatory strategies adopted to stimulate the preparation of clearer and shorter scientific reports.

Despite the methodological limitations, important elements in the experimental designs were correctly identified in our survey, contributing to the reliability and reproducibility of the studies, especially in the most recent reports. Data such as the animal model, sex, weight, parasite strain, route of administration and parasitaemia were consistently described. Our results show that murine models were most used in the investigations. A suitable selection of animal species and genetic background is crucial in investigations of parasitic diseases, since these factors are directly related to host resistance and susceptibility to the pathogen (Andrade *et al.*, 2002; León *et al.*, 2017). In the present study, the presence of *T. cruzi* infection was associated with a high prevalence of *T. cruzi* infection. In addition to the similarity with humans, murine models are easier to handle, lodge and present low maintenance costs compared with other animal models. Our data also revealed that only a reduced number of studies used larger animals as models of Chagas' disease, especially horses and pigs. Possibly this limitation was due to the low availability, high costs and problems to attain the necessary approval by the ethics committees.

Most studies used similar strains to induce *T. cruzi* infection. The selection of the parasitic strain is essential because they vary in infectivity, pathogenicity, tropism and virulence (Andrade *et al.*, 2002; Manoel-Caetano and Silva, 2007; León *et al.*, 2017). Most of the strains used in the studies analysed in the present work are known to present high virulence and

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pathogenicity. These data are in accordance with the main morphological findings presented in our results, with a predominance of moderate-to-intense inflammatory foci, and a high number of mononuclear and lymphocytic infiltrates. These elements are closely correlated with acute patterns of infection since the animals often die before developing chronic infection (Chatelain and Konar, 2015). Because the strains of parasites used matched the phases of interest in Chagas' disease, i.e. the acute phase, the studies analysed herein exhibited an important element of methodological consistency, with a positive effect on the validity of the description.

The most frequent morphological findings found in our review were foci of inflammatory infiltrates, predominantly of mononuclear cells, mainly lymphocytes (CD4 + T and CD8 + T), in the CNS during the acute phase of *T. cruzi* infection. The sites most frequently identified with inflammatory foci were perivascular spaces, meninges of the brain, and the nerve roots of the spinal cord. Considering that the CNS is thought to be an immunoprivileged site due to the presence of the BBB (Ziv *et al.*, 2006), the development of inflammatory infiltrates in these regions only occurs in cases of intense infection, especially in cases of American or African trypanosomiasis (Galea *et al.*, 2007; Masocha and Kristensson, 2012). This may explain why the way *T. cruzi* manages to enter the CNS is poorly studied. Increased BBB permeability occurs when factors derived from pathogens (e.g. cysteine protease) are recognized by T lymphocytes. The activation of these lymphocytes leads to the production of cytokines (IFNα/β, IFNγ and TNFα), which diffuse into the CNS, thereby stimulating the brain endothelial cells to produce Activated Leucocyte Adhesion molecules (ALCAM, ICAM-1) and Vascular Cell Adhesion-1 molecules (VCAM-1) that favour cell migration. In addition, these cytokines also stimulate astrocytes to produce chemotactic cytokines such as CXCL10 that increase the permeability of the BBB, allowing the dissemination of flagellate forms of *T. cruzi* and also of lymphocytes that may contain within them the amastigote form of the parasite (Rocha *et al.*, 1994; Silva *et al.*, 2010; Masocha and Kristensson, 2012). In our study, the presence of amastigotes in the cytoplasm of basal, glial (astrocytes, microglia, ependymocytes and oligodendrocytes) and Purkinje cells, as well as in the cerebellum was observed in most studies, along with the foci of inflammatory lymphocytic infiltrates in the CNS. On the other hand, the presence of flagellate trypanosomes also stimulates the humoral response and consequently increases the permeability of the BBB (Masocha and Kristensson, 2012).

Moreover, mononuclear cells and macrophages respond by recognizing circulating Pathogen-Associated Molecular Patterns (PAMPs), thereby producing proinflammatory cytokines, such as IL-1 and IL-6, which diffuse into the CNS and stimulate the production of mediators, such as prostaglandin E, that increase vascular permeability and consequently facilitate the entry of inflammatory cells into the CNS (Vitkovic *et al.*, 2000; Banks, 2009; Chizzolini and Brembilla, 2009; Kawai and Akira, 2010; Guillamón-Vivancos *et al.*, 2015). All these alterations allow the installation of an inflammatory process that will be controlled by astrocytes, microglia and neurons (Galea *et al.*, 2007). However, the mechanisms underlying this control and which mediators are involved in inhibiting cell proliferation remain unclear. It is now known that regulatory T cells are also activated to control cell migration and consequently inflammation (Trajkovic *et al.*, 2004). However, in the case of *T. cruzi* infection, this modulation is not sufficient to prevent cell migration and consequently to limit the installation of acute inflammation in the tissue (Cabral-Piccin *et al.*, 2016).

The various clinical manifestations that occur throughout the development of Chagas' disease are directly related to the genotype of the circulating parasites, the geographic origin and the cycles of wild and domestic transmission. This is because these variations in the populations determine the tropism to the tissues, the parasitaemia, and the pathogenesis in the vertebrate hosts during the acute and chronic phase of the disease (Andrade *et al.*, 1999; Macedo *et al.*, 2004; Magalhães-Santos *et al.*, 2004). In our review, we observed that, after 50 inoculations with more than 20 different *T. cruzi* strains, those belonging to the TcI (ex Colombian), TcII (ex Y) (Galea *et al.*, 2007) and TcIV (ex AM05) (Meza *et al.*, 2014) were those that presented histotropism for the CNS. The TcI and TcII strains can be found in other tissues (Andrade *et al.*, 2010; Galea *et al.*, 2007; Zingales *et al.*, 2012), although the TcIV genotypes favour CNS tropism (Meza *et al.*, 2014). This trend shows us the importance of knowing the genotype of *T. cruzi* to fully understand the manifestations and clinical evolution of the disease. Based on this tropism, it is possible to evaluate the need for new, more efficient and less toxic treatments according to the main infection sites of the parasite. The relationship between the parasite genotype and tropism may be relevant for the rational design of drugs capable of reaching the priority infection sites. However, there is a natural difficulty in the treatment of infections in the CNS, because the BBB is a highly selective component that limits the therapeutic distribution, making it difficult to use effective concentrations for parasitism in the nervous tissue without causing toxic effects to the organism. Due to this real difficulty, some groups are dedicated to the study and development of new drugs effective and with low side effect (Flores-Vieira and Barreira, 1997; Flores-Vieira *et al.*, 1997; Jeganathan *et al.*, 2010; Perin *et al.*, 2017).

Histopathological analysis was the most used strategy to study morphological changes in the CNS during *T. cruzi* infection, most probably because it is a simple, fast and economical method when compared with electronic microscopy and immunohistochemistry analysis. The method allows the study of large sections of the tissue sample and provides a valuable diagnostic tool to examine the internal architecture of the infected tissues (Mescher, 2016). In addition, histopathological studies allow the identification of typical tissue responses that vary as the infection progresses from the acute to chronic or disseminated phases (Gupta *et al.*, 2009). The most great challenge for the real comprehension of the pathogenesis of the nervous clinical form of Chagas disease is the lack of association between the morphological/histopathological lesions and the clinical manifestations of patients. When histological changes observed in tissues have a direct relevant relationship with the clinical manifestations, and can thus provide complementary information to correctly identify some particular type of microorganism that may be causing of alteration in tissues (Woods and Walker, 1996; Procop and Wilson, 2001). Therefore, the analysis of studies that report specific morphophysiological changes caused by parasites or a particular strain of the parasite may contribute to the association between tissue/physiological changes and the clinical picture manifested by individuals with parasitic diseases, which may help to make a diagnosis and treatment more efficient.

This review is the first to systematically compile the results of studies describing the changes caused by *T. cruzi* in the CNS. Our findings reinforce the importance of some analyses in the early stages of the diagnosis of Chagas' disease, such as parasite load, since in some cases the surrounding parasites may not be detected, but may be causing progressive damage to organs such as the heart, oesophagus and colon (Gironès and Fresno, 2003; Teixeira *et al.*, 2006). This negative correlation is due to critical aspects of Chagas' disease such as the genotype and the infecting strain of *T. cruzi* as well as the host's immunogenetics (Costa *et al.*, 2009), which would dictate the final predictive parameters. Thus, the parasite's persistence mechanisms and the

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quality of the immune response may determine the extent of tissue damage (Gutierrez *et al*., 2009). Based on this, we described herein the organs or tissues that can undergo alterations and the type of alterations, which may help an accurate description of the clinical picture associated with the disease. Although this study evaluated only animal models and does not necessarily accurately reflect human disease, it addresses clinically relevant issues, including tissue tropism, symptoms, immune response and treatments (Chatelain and Konar, 2015), and therefore may have its results extrapolated to human chagasic patients.

The selection of the studies composing this review was based on widely accepted and recommended practices for systematic reviews. A relevant issue highlighted in our study is the bias of the publications. To detect this, we used the ARRIVE Guidelines (Kilkenny *et al*., 2010), which allow to test the degree of reliability of the studies individually and later collectively. It allowed us to notice that various aspects related to the organization and description of the experiments were neglected, among them the lack of randomization and the absence of double-blind studies, mainly in studies performed more than 15 years ago. Our data suggest a low methodological rigor of the studies at the beginning of the research efforts involving *T. cruzi*. For this reason, a systematic review on this subject is important, since it indicates the shortcomings of the work already carried out and indicates that future work should be more careful to allow the reproducibility of the techniques and the quality of the results.

In conclusion, the present systematic review was able to compile studies that evaluated histopathological changes in the CNS during *T. cruzi* infection, in which the differential tropism of the TcI, TcII and TcIV and TcVI genotypes was evidenced by structures of the brain, cerebellum and spinal cord. Changes such as the intensity of the inflammatory foci and the number of nests of parasites were shown to be linked to the genetic diversity of the different strains of *T. cruzi*, geographic origin and cycles of wild and domestic transmission of the strains. Finally, we highlight how detailed knowledge about the various clinical conditions that may occur during Chagas' disease are determinant not only to support the current knowledge about this disease but also as a facilitator of early and efficient diagnosis to guarantee an adequate treatment and a good quality of life for the individuals affected.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0031182019000210

Author ORCIDs. Rômulo Dias Novaes, 0000-0002-3186-5328; Reggiani Vilela Gonçalves, 0000-0002-5831-3590

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