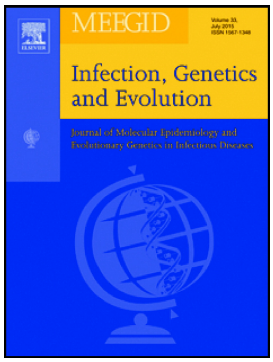

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Human infectiousness and parasite load in chronic patients seropositive for *Trypanosoma cruzi* in a rural area of the Argentine Chaco

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Abstract

A key parameter in the transmission of vector-borne infections, including Chagas disease, is the ability of the different host species to transmit the parasite to the vector (infectiousness). Here, we determined infectiousness to the vector of *Trypanosoma cruzi*-seropositive humans examined by artificial xenodiagnosis (XD), established its relationship with *T. cruzi* DNA levels

(a surrogate of intensity of parasitemia) quantified by real-time PCR (qPCR), and assessed whether infectiousness was associated with the body mass index (BMI), age, ethnic background and parasite genotype. XD was performed to 117 *T. cruzi*-seropositive residents from Pampa del Indio and parasite load was quantified in 81 of them. By optical microscopy (OM) 32.7% of the people were infectious and this fraction doubled (60.5%) when XD triatomines were examined by molecular methods. The mean infectiousness (defined as the percentage of infected triatomines among the total number of insects examined by OM 30 days post-feeding) was 5.2%, and the mean parasite load was 0.51 parasites equivalents per ml. Infectiousness to the vector was associated negatively with age and BMI, and positively with the detection of parasitemia by kDNA-PCR, and parasite load by qPCR in univariate analysis. Patients with a positive XD by OM exhibited a significantly higher mean parasite load. Using multivariate regression, infectiousness was associated with parasite load (positively) and with the household presence of *T. infestans* and Qom ethnic group (negatively); no significant association was observed with age or its interaction with ethnicity. Infectiousness was aggregated: 18% of the people examined by XD generated 80% of the infected triatomines. Detecting and treating the super-infectious fraction of human hosts would improve their prognosis and disproportionally impact on domestic transmission risks.

Keywords: Xenodiagnosis; *Triatoma infestans;* Parasite load; Ethnicity; *Trypanosoma cruzi*

1. Introduction

Chagas disease is a vector-borne Neglected Tropical disease (NTD) which affects millions of people worldwide; nearly 70 millions are at risk in the Americas (WHO, 2015). *Trypanosoma cruzi,* its etiological agent, is mainly transmitted by triatomines (Hempitera: Reduvidae), and in Argentina the main vector is *Triatoma infestans* (Dias et al., 2002; Schofield et al., 2006). Vector control efforts have strongly reduced its geographical distribution in the Southern Cone of South America and interruption of domestic transmission mediated by *T. infestans* has been

accomplished in Chile, Uruguay, Paraguay, Brazil and parts of Argentina and Bolivia (OPS / WHO, 2018). However, domestic infestation and parasite transmission mediated by *T. infestans* are still present in vast areas of Bolivia, Argentina and southern Peru.

Infectiousness to the vector is a key parameter related to parasite transmission. Despite the broad range of mammal hosts that can be naturally infected with *T. cruzi*, their infectiousness (defined as the percentage of uninfected insects that become infected after blood-feeding once on an infected host) varies widely among species (Gürtler and Cardinal, 2015). Human infectiousness is generally low compared to other non-human species. For example, dog infectiousness (ranging between 27.5-62.3%) is 10 times higher than that of chronic humans (mean = 4.4%) (Gürtler y Cardinal, 2015). Moreover, infectiousness of seropositive humans is widely variable among studies, ranging from 2.6% (Gürtler et al., 1996) to >50% (Schenone et al., 2000) (Table S1). This variability is in part due to methodological differences (number, stage and species of triatomine employed; artificial or natural tests) as well as targeting different groups (children, adults, acute or chronic Chagas disease cases, immunocompromised patients) (Braz et al., 2001; da Cruz Oliveira et al., 1997; dos Santos et al., 1995; Sartori et al., 2002; Schenone, 1999; Teixeira de Freitas et al., 2011). An additional source of variability is the patients' epidemiological context (current or past), which in most of these studies was ill-defined since they included patients attending hospitals.

Recent studies have focused on the relationship between parasitemia and infectiousness to the vector and their impact on transmission of leishmaniosis (Courtenay et al., 2014; Miller et al., 2014; Seblova et al., 2013; Silva et al., 2016), malaria (Slater et al., 2019) and Chagas disease (Enriquez et al., 2014; Saavedra et al., 2016). Infectiousness is affected by parasitemia since the probability that a triatomine becomes infected after a single bloodmeal depends on whether there are (and the amount of) circulating parasites in the blood (Moll-Merks et al., 1988). However, the quantitative relationship between infectiousness and parasite load is still

unclear. *T. cruzi* parasitemia in humans ranged from 0 to more than 10,000 parasites per ml (Bua et al., 2013), and the expected parasite load might depend on several factors described below. Whether parasitemia values are considered low or high depends on the particular study population and its peculiarities.

Trypanosoma cruzi parasitemia is controlled by the host's immune system, which may be affected by factors related to the host and the parasite: age, disease phase, nutritional state (Muñoz et al., 2004; WHO, 2000), parasite genetic makeup, and co-infections with other etiological agents (Bustos et al., 2019). Higher parasite loads have been found in people with acute *T. cruzi* infection compared to chronic infections (Ramírez et al., 2015), and infants congenitally infected exhibited parasitemia several orders of magnitude higher than that of their respective mothers (Bua et al., 2013, 2012; Virreira et al., 2007). People co-infected with HIV and *T. cruzi* presented elevated parasitemias (Burgos et al., 2005; Teixeira de Freitas et al., 2011), whereas immunocompetent chronic patients usually have low parasitemia (Melo et al., 2015). In children up to 18 years of age, a negative relationship was observed between parasitemia and age (Duffy et al., 2009). No differences were found in parasite load among T. cruzi genotypes (Ramírez et al., 2015). Immune response boosted by reinfections may explain why pregnant women who lived in areas under vector surveillance exhibited higher parasitemia than those who lived in areas with active vector-borne transmission (Rendell et al., 2015) or exhibited higher congenital transmission rates (Sanchez Negrette et al., 2005). Variations in parasitemia were explained by circadian rhythm variations in several parasitic diseases (Rijo-Ferreira et al., 2017). However, no circadian rhythm has been found in *T. cruzi* so far (Castro y Prata, 2000).

Here, we studied a well-defined human population from the Argentine Chaco to: i) determine infectiousness to the vector and parasite load, ii) establish the quantitative relationship between them, and iii) assess whether infectiousness is associated with age,

gender, ethnicity, body mass index, domestic infestation with *T. infestans* (as a proxy of exposure to reinfection during the lifetime of the person) and parasite genotype. Based on previous knowledge, we predicted that: i) infectiousness, measured by artificial xenodiagnosis, and parasite load, quantified by qPCR, would be low since all study individuals were in the chronic phase of infection, and ii) infectiousness would increase with parasite load given that human infectiousness rarely reaches 100%.

2. Material and Methods

2.1. Study area

Fieldwork was conducted in the rural area of Pampa del Indio Municipality (25º55'S 56º58'W), Chaco Province, Argentina. The rural area was subdivided for operational reasons in sections named I-IV and houses were georeferenced. The study area has been under vector surveillance since 2007-2008, when a community-wide spraying with residual pyrethroids insecticides occurred. Annual or bi-annual post-intervention surveys aiming at full coverage of rural houses were conducted (Gaspe et al., 2015; Gurevitz et al., 2011; Provecho et al., 2017). Of the two local ethnic groups, Qom and Creole, Qom people represented 79% of the rural population.

2.2. Study population

A total of 3,216 residents of Areas II, III and IV were serodiagnosed for *T. cruzi* infection using standardized procedures (Sartor et al., 2017) between 2012 and 2017. Additional serodiagnosis was performed to a few inhabitants from a neighboring area and periurban Pampa del Indio. Seropositive individuals below 21 years old were eligible for etiological treatment and referred to the local hospital "Dr. Dante Tardelli". At the time of performing routine tests prior to etiological treatment, a 2 ml aliquot of blood was mixed with an equal volume of Guanidine-EDTA Buffer (GEB) for molecular diagnosis of *T. cruzi* infection, and 3 ml

of blood were mixed with heparin for artificial xenodiagnosis. In order to include people > 21 years of age, 5 ml of blood were additionally extracted in some adults who self-reported a previous seropositive result during two serosurveys conducted between 2014 and 2015; samples were processed as mentioned above.

2.3. Artificial xenodiagnosis

Xenodiagnosis tests were performed using 20 fourth–instar nymphs of laboratory-reared *T. infestans* (kept unfed for at least 3 weeks) as described by Macchiaverna et al. (2018). For humans, artificial xenodiagnosis is preferred to classic xenodiagnosis since triatomines feed on freshly extracted blood, and direct contact with the person is avoided (Castro et al., 2004; dos Santos et al., 1995). The time elapsed between blood extraction and the onset of feeding was < 5 min. Bug feces were analyzed by optical microscopy (OM) at 400× 30 and 60 days post-feeding to determine the infection with *T. cruzi*. Molting and mortality rates by 30 days after feeding were used as indices of xenodiagnosis quality (Gürtler et al., 2007).

A total of 161 artificial xenodiagnosis were performed between 2013 and 2016 at the local primary healthcare posts or rural schools, with 125 (78%) applied to *T. cruzi*-seropositive residents. During the performance of 5 XD, the latex membrane that separates the blood from the triatomines broke and the triatomines could not feed; these XD were discarded from analyses. In addition, a XD was performed on a young woman who later indicated that she had recently completed etiological treatment; given that all the examined triatomines resulted OM-negative, this patient was excluded from the analyses. In total, 119 of the 161 XD performed were analyzed in this study.

2.3.1.Infectiousness

We defined a person as infectious if at least one *T. infestans* was infected with *T. cruzi* among those examined by OM. Infectiousness was calculated as the percentage of *T. infestans*

infected with *T. cruzi* divided by the number of live insects examined for infection at 30 days post-feeding.

In order to evaluate the percentage of false-negative triatomines by OM, a kDNA-PCR diagnosis was performed extracting DNA from the rectal ampoules of the triatomines that were not infected by OM (Enriquez et al., 2014). Up to 5 OM-negative *T. infestans* were randomly selected from each XD; in total, 527 triatomines were tested by kDNA-PCR.

2.4. Molecular diagnosis from GEB

The 4ml GEB-blood samples were stored at 4ºC until use. Samples were boiled for 10 minutes in order to liberate the catenated minicircles and distribute the target sequences homogeneously in the blood sample (Britto et al., 1993). An aliquot of 300 µl was mixed with 3 ng of an internal amplification control DNA (IAC) and used for DNA extraction using commercial purification columns (Qiagen).

The presence of *T. cruzi* was determined qualitatively by a kDNA-PCR amplification of the 330pb fragment of the minicircle of the kinetoplast (Burgos et al., 2005). Bloodstream parasite load was quantified by real-time PCR (q-PCR) targeting the *T. cruzi* satellite DNA (Sat-DNA), a conserved region of the parasite genome (Duffy et al., 2009). Seropositive individuals with a positive kDNA-PCR and/or positive XD were selected for parasite load quantification. Parasitemia was measured as parasite equivalents per ml (Pe/ml) as described in Bua et al. (2012). To verify that DNA extraction was correct, a fragment of the 289bp human β-actin gene in all GEB samples was amplified (Velázquez et al., 2014). IAC amplification by qPCR was used as a measure of efficiency of the DNA extraction step (Bua et al., 2012)

2.5. DTU identification

Parasites were isolated from feces of *T. cruzi*-infected *T. infestans*. DTUs were identified from the parasite isolates using two different PCR protocols as reported in Macchiaverna et al. (2018).

2.6. Data analysis

Agresti–Coull binomial 95% confidence intervals (CI) were used for proportions. Fisher's exact tests and pairwise Wilcoxon tests were used to compare proportions of infectiousness and mean parasite load, respectively, between identified DTUs and XD results. The Kappa index was used to evaluate the concordance between XD and kDNA-PCR from GEB samples. A Spearman correlation was assessed between infectiousness and parasite load. In order to evaluate factors potentially associated with infectiousness, GLMs were implemented in the R environment (Team R, 2018, version 3.3.5), using the packages MuMIn (Barton, 2016), ResourceSelection (Lele et al., 2014) and car (Fox y Weisberg, 2011). Infectiousness was the response variable, and a binomial distribution with logit link function was considered. Univariate and multivariate models were performed. Due to the frequency of missing data for parasite load, two multivariate models were run using different datasets:

Model 1: Infectiousness ~ Age + Gender + Ethnicity + Presence of *T. infestans* + Age x Ethnicity

Model 2: Infectiousness ~ Age + Gender + Ethnicity + Presence of *T. infestans* + Age x Ethnicity + Parasite load

For model selection, all models which had a ΔAIC smaller than 2 were selected, and OR and CI were estimated averaging over the selected models (Burnham y Anderson, 2002).

The Body Mass Index (BMI), a simple surrogate of nutritional state, was classified as low if it was less than 18.5, normal between 18.5 and 25, and high if it was greater than 25 as recommended by WHO, 2000. For BMI calculation age and gender were considered. Since weight and height were only registered for approximately half of the patients, BMI was excluded from multivariate analysis.

The presence of *T. infestans* was a categorical variable with 3 levels: absence of *T. infestans*, presence of *T. infestans*, or presence of *T. cruzi*-infected *T. infestans*. It combined the finding of *T. infestans* in domiciles, kitchens or storerooms of each household during any vector survey between 2008 until the date of the XD, and the report on whether each person

had ever lived in a house infested with triatomines. Although validity differs between these different infestation and vector-borne transmission risk assessments, our intention was to score previous risk, occurring before the vector control activities were launched in the study area. For children born after the onset of the vector control program (i.e.: ≤8 years of age), the date of birth and the timing when the *T. infestans* was captured during any survey were considered. Infection with *T. cruzi* was determined by OM examination of a subset of the *T. infestans* collected (Provecho et al., 2017).

Age (in years) and parasite load (parasite equivalents/ml) were considered continuous variables; ethnic group was a categorical variable with two levels (0: creoles, 1: Qom and mixed ethnic background).

Multicollinearity was assessed by calculating the Variance Inflation Factor (VIF) and the condition numbers for the explanatory variables. The overall quality of the fitted logistic regression models was assessed using the Hosmer-Lemeshow test.

2.7. Ethical statement

The procedures for human serodiagnosis and treatment (Protocol N° TW-01-004) and parasite diversity tests were approved by the "Comité de Ética en Investigación Clínica" (Ethics Committee in Clinical Research) of Buenos Aires, Argentina. Each person, or their parents or guardians (for minors), signed an informed consent prior to venipuncture.

3. Results

3.1. Artificial xenodiagnosis

A total of 119 xenodiagnoses were performed to 117 seropositive inhabitants (2 siblings were examined twice on different occasions). The majority of the XDs were performed to women (58.8%), who represented 46.0% of the study population. The median age of XD-examined people was 15 years (Q1-Q3 = 12-29), ranging from 5 to 73 years. Most people analyzed (76.5%) belonged to the Qom ethnic group (Table 1). The observed mortality rate of

triatomines at 30 days post-feeding was 8.4% (95% CI = 7.3-9.6%), and the bug molting rate was 10.8% (95% CI = 9.6-12.2%, Table 1).

3.2. Infectiousness to the vector

Of the 119 XD performed, 39 (32.8%, 95% CI = 25.0-41.7) were positive by OM. Another 35 XD resulted positive by complementing the diagnosis with kDNA-PCR of triatomine rectal contents. Combining both techniques, 72 XD (60.5%; 95% CI = 51.5-68.8) were positive and 47 were negative (Figure 1a). We identified 111 infected triatomines as determined by OM, while another 68 were found infected by kDNA-PCR (Figure 1a).

The mean infectiousness by OM was 5.2% (95% CI = 4.3-6.2, n = 2167). The percentage of false negatives estimated by OM compared to kDNA-PCR was 13.1% (95% CI = 10.5-16.3, n = 527). In positive XD, the mean number of infected triatomines by OM was 2.9 per person (SD = 2.3), and the mean was 2.5 (SD = 2.2) for both diagnostic methods combined (OM and kDNA-PCR). The mean number of infected *T. infestans* for all XDs was 1.0 (SD = 1.9) when only OM results were considered, but when OM and kDNA-PCR diagnosis were combined the mean rose to 1.5 (SD = 2.1) infected triatomine per person. Infectiousness to the vector was aggregated: 18% of the people examined by XD presented 80% of the infected triatomines.

3.3. Molecular diagnosis and determination of parasite load

We analyzed 121 GEB from seropositive people. Forty-five samples were positive using kDNA-PCR (37.2%, 95% CI = 29.1-46.1), and 76 did not amplify the target DNA. All samples amplified for human β-actin. For 107 individuals with both xenodiagnosis and kDNA-PCR, the kappa index showed a slight agreement (k = 0.20) between techniques (Table 2).

Eighty-one GEB samples were analyzed by qPCR; the mean parasite load was 0.51 Pe/ml (SD = 0.79), ranging between 0 - 4.21 Pe/ml (Figure 1b). Some samples (n=7) exhibited undetectable *T. cruzi* DNA, and 26 were under the cutoff; these samples were assigned a value

of 0 Pe/ml. Therefore, 48 samples (59.2%) had a detectable and quantifiable parasitemia level by qPCR. Excluding samples with nil parasitemia, the mean parasite load was 0.78 (SD=0.87) (Figure 1b).

3.4. Factors associated with infectiousness

In univariate analyses, infectiousness to the vector was negatively and significantly associated with age and BMI, and positively with the detection of parasitemia by kDNA-PCR and parasite load (Table 3).

In the first dataset (model 1) including demographic variables, the presence of *T. infestans* and all individuals with XD (n = 117), a marginally significant interaction was found between ethnicity and age; infectiousness decreased with age, but with different slopes according to the host's ethnic background (Table 4). In model 2, infectiousness was positively associated with parasite load and negatively and significantly associated with the presence of *T. infestans* (Table 4). From the multimodel analysis, 3 top models were obtained for the first dataset while only one model was selected for the second (Table 5). The explained variance was 15.1% and 28.7% for model 1 and 2, respectively. The models had a good fit to the data (Hosmer-Lemeshow goodness of fit test, p= 0.93 and p= 0.99 for model 1 and model 2, respectively).

Infectiousness increased with parasite load (Table 4), and maximum infectiousness (44%) was at 1.5 Pe/ml (Figure 2). A highly significant positive correlation between infectiousness and parasite load was observed (Spearman correlation ρ=0.45, p<0.001).

People with a positive XD by OM had a higher mean parasite load (mean = 0.78, SD = 0.91) than those with a negative XD by OM and a positive XD by kDNA-PCR (mean = 0.30, SD = 0.66) (Wilcoxon test, p = 0.008) and also than those with a negative XD as determined by both methods (mean = 0.29, SD = 0.65) (Wilcoxon test, p = 0.02, Figure 3). No difference in mean

parasite load was observed between individuals with a positive XD only by kDNA-PCR and with negative XD by both OM and kDNA-PCR (Wilcoxon test, p = 0.6).

No significant differences in infectiousness (17.0%, 7.6%, 16.6%; Fisher's test, p = 0.2) or mean parasite load (0.81, 0.10, 0.92; Wilcoxon test,, p = 0.3) were observed between the DTUs identified, TcV, TcVI or TcV and/or TcVI, respectively (Figure 4). We were not able to identify the infecting DTUs from xenodiagnosis bugs PCR-positive only due to the low concentration of parasite DNA.

4. Discussion

By means of artificial xenodiagnosis and qPCR, this study shows that the inhabitants of these rural endemic communities in the Argentine Chaco presented low infectiousness to the vector (mean= 5.2%) and a very low parasite load (mean = 0.5 Pe/ml). Artificial xenodiagnosis allowed us to estimate the transmission capacity of human hosts; we found that 32.8% of the examined seropositive people were infectious to *T. infestans* by OM, and this percentage almost doubled when molecular diagnosis was added. This value exceeds previous findings by OM which ranged between 7.4-35.6% (Braz et al., 2001; Gürtler et al., 1996; Junqueira et al., 1996; Sartori et al., 2002; Teixeira de Freitas et al., 2011) but is similar to the infectious percentage (57.9%) reported from a similar study in Chile (Coronado et al., 2006). Integrating these results with vector-host contact rates will evaluate the contribution of chronic infected humans as sources of *T. cruzi* in domestic transmission cycles.

In artificial XD, bloodstream parasites used in the artificial feeder could die during the process and therefore infectiousness be underestimated (Profeta de Luz, 1999). Nonetheless, if performed adequately similar results can be obtained between classic and artificial human XD (Panameño Pineda et al., 1998). The same artificial feeding device was used previously and similar results in natural and artificial human XD were obtained (Cardinal et al., 2008). Also, the infectiousness found in this study was slightly higher than those obtained in natural

xenodiagnoses applied to humans (Cardinal et al., 2008; Castro et al., 2004; dos Santos et al., 1995; Gürtler et al., 1996; Teixeira de Freitas et al., 2011). These might indicate that if there was any mortality of parasites due to handling procedures this might have been negligible. Moreover, the quality of the XD performed was evidenced by similar bug mortality and molting rates in the artificial XD versus those used in classic XD of dogs, cats and wild animals (3-6% and 8-15%, respectively) (Enriquez et al., 2014; Alvarado-Otegui et al., 2012; Orozco et al., 2016).

The mean parasite load was 0.51 parasite equivalents per ml and its range was between 0-4.21. These values are similar to those reported in a neighboring rural area of Pampa del Indio (Sartor et al., 2017) and in chronic humans seropositive for *T. cruzi* (Alvarez et al., 2016; Bua et al., 2012; Duffy et al., 2013; Lucero et al., 2016; Melo et al., 2015; Ortiz et al., 2012). Similarly, 37% out of 100 chronic patients exhibited parasitemia ≤ 1 parasite equivalent per ml in Chile (Saavedra et al., 2016). None of the study individuals had the high parasite loads observed in newborns with congenital infection (Bua et al., 2013), or people co-infected with HIV (Teixeira de Freitas et al., 2011). Parasite load was positively associated with infectiousness to the vector, and patients with a positive XD by OM exhibited a higher mean parasite load than those with a negative XD by OM, regardless of the kDNA-PCR results, as observed elsewhere (Saavedra et al., 2016).

Age was negatively associated with infectiousness to the vector, and a marginally significant interaction with ethnicity and age was observed. The occurrence of a positive xenodiagnosis decreased with age (Gürtler et al., 1996; Hoff et al., 1979; Schenone et al., 1995). Age was also negatively associated with parasite load, but the study population only included children under 18 years old, and no other factors were considered (Duffy et al., 2009). How the immune response varies with age and may modulate parasitemia was not considered in this study.

In multivariate analyses, the report of the household presence of *T. infestans* during the lifetime of the person (a proxy for reinfection probability) was a significant protective factor. This variable combined self-reported triatomine exposure, which is subject to recall bias, and our own findings of triatomines during the surveillance phase (Gaspe et al., 2018; Provecho et al., 2017). Similarly, pregnant women living in areas under vector surveillance in Bolivia exhibited higher parasitemia than those living in areas with active transmission (Rendell et al., 2015). These results are consistent with the hypothesis that reinfections would boost host immune response, and limit *T. cruzi* parasitemia. On the contrary, reinfections were associated with dogs' infectiousness (Gürtler et al., 1992) and with a slight increase of *T. cruzi* parasitemia in experimentally inoculated dogs and mice (Bustamante et al., 2007; Machado et al., 2001). However, the absence of association was also observed (Enriquez et al., 2014; Gürtler et al., 2007). These discrepancies may be explained by the different metrics used to assess reinfection probabilities, their different validity and the difficulty to define when the exposure occurred.

A low BMI might indicate a poor nutritional state that can affect the immune system and its capacity to control *T. cruzi* infection or other pathogens (WHO, 2000). The BMI was negatively associated with infectiousness to the vector in univariate analysis. Unfortunately, this variable presented a high number of missing data and could not be incorporated into the multivariate analysis. In dogs, an increase in infectiousness to the vector and *T. cruzi* parasitemia was observed in animals with the worst body conditions (Enriquez et al., 2014).

Ethnicity was not significantly associated with infectiousness in this study, though a marginally significant interaction with age was observed. Ethnic background may represent poverty-related or other social-economic factors rather than a biological background. Domestic infestation risk, vector abundance and triatomine infection with *T. cruzi* were associated with high vulnerability conditions in a neighboring study area (Fernández et al.,

2019), and *T. cruzi* infection risk and social vulnerability were unequally distributed among ethnic groups. If reinfection chances affect *T. cruzi* parasitemia (Rendell et al., 2015), the Qom population may have experienced this effect.

The XD protocol is highly laborious and requires an experienced operator. However, its main advantage is that it allows obtaining parasite isolates from rural residents without mobilizing them to a hospital or other establishment with the necessary infrastructure to perform hemoculture (Profeta de Luz, 1999). Human *T. cruzi* isolates were used to determine the infecting DTU (Macchiaverna et al., 2018), and to eventually correct parasite loads for the infecting DTU since TcI presents 10 times less copies of the satellite DNA than TcVI (Duffy et al., 2009). Parasite DTUs identified in the inhabitants of Pampa del Indio were the hybrid TcV and/or TcVI. We did not find differences in their infectiousness to the vector or parasite load, similar to previous findings (Ramírez et al. 2015). In contrast, TcI-infected dogs and cats from the study area had nil infectiousness despite having moderate parasite loads (Enriquez et al., 2014). Since human DTU identification was achieved from *T. cruzi*-.infected triatomines used in XD, TcI-infected humans may have been lost if the same pattern occurred. Also, TcI displayed differential tropism in humans further masking the chances of detecting them in venous blood (Burgos et al., 2008). Unfortunately, DTU identification from human GEB samples was unsuccessful, probably due to the very low parasite loads registered.

Our study has strengths and limitations. A wide range of ages was encompassed, and a well-defined population was studied. The procedure for performing artificial xenodiagnoses was standardized, which allowed making comparisons between surveys. However, a limitation was the low volume of blood employed, which was not enough to feed triatomines until repletion in most cases. It was not feasible to draw more blood for operational reasons, introducing a difference in the engorgement level of triatomines attained in classic or artificial XD.

The "80/20" rule (Woolhouse et al., 1997) accounts for the typical aggregated distribution of parasites, where 20% of hosts usually harbor 80% of the net transmission potential. A concept related to this empirical rule is the presence of "super-spreader" hosts (Stein, 2011). The study population had an aggregated distribution of infectiousness since 18% of the 117 people analyzed by xenodiagnosis generated 80% of the infected triatomines and could be considered super-infectious. These super-infectious individuals with infectiousness higher than 40% were four Qom boys aged ≤12 years who cohabited with at least two other infected people.

Social vulnerability was linked to human seroprevalence of *T. cruzi* and transmission risk (Fernández et al., 2019). How BMI is distributed among the social groups and whether a better nutritional state would modify infectiousness to the vector is unknown. Detecting and treating the super-infectious fraction of human hosts would improve their prognosis and also disproportionally impact on domestic transmission risks if domestic reinfestation occurs.

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Table 1: Summary values of artificial xenodiagnosis performed to *T. cruzi*-seropositive inhabitants, Pampa del Indio, 2013-2016.

Live triatomine at 30 days post feeding	2167
Live triatomine at 60 days post feeding	1194
Molted triatomines at 60 days post feeding (%)	235 (10.8%)
Dead triatomines at 30 days post feeding (%)	200 (8.4%)
Dead triatomines at 60 days post feeding (%)	612 (25.9%)
Women with XD	70 (58.8%)
Qom patients with XD	91 (76.5%)

Table 2: Diagnosis of *T. cruzi* infection by kDNA-PCR from Guanidine-EDTA blood samples from chronic seropositive people and comparison with the result obtained by optical microscopy examination of artificial xenodiagnosis bugs, Pampa del Indio, 2013-2016

	XD by OM		Without XD	Total
kDNA-PCR	Positive	Negative		
Positive	17	24	4	**45**
Negative	15	51	10	**76**
Without GEB sample	7	3	-	**10**
Total	**39**	**78**	**14**	**131**

Table 3: Univariate risk factor analysis of infectiousness to the vector. N is the number of observations for each variable.

Variable	**N**	**OR (95% CI)**	***P* value**
Age	117	0.94 (0.91 - 0.96)	**<0.001**
Ethnicity	117		
Creole		1	
Qom		0.81 (0.53 - 1.26)	0.33
Gender	117		
Female		1	
Male		1.09 (0.74 - 1.60)	0.66
BMI	59	0.93 (0.86 - 0.99)	**0.04**
Parasitemia detected by kDNA-PCR	107		
Negative		1	
Positive		2.91 (1.84 - 4.69)	**<0.001**
Parasite load	70	1.06 (1.04 - 1.08)	**<0.001**
Presence of *T. infestans*	117		
No		1	
Yes and not infected, or ND*		0.69 (0.45 - 1.10)	0.11
Yes and infected		1.7 (0.90 - 3.13)	0.10

*This category combines infested houses with uninfected bugs or infested houses with no data on infection status of the collected bugs.

Table 4: Multiple logistic regression for human infectiousness. Model 1 (N=117) and model 2 (N=70). Odd Ratio (OR), Confidence intervals (CI), *P* value and Relative importance (RI) are informed for each variable using the averaged model.

	Model 1			**Model 2**		
Variables	**OR (95% CI)**	***P* value**	**RI**	**OR (95% CI)**	***P* value**	**RI**
Age	0.96 (0.92 - 1.00)	**0.05**	**1.00**	1.00 (0.97 - 1.03)	0.83	0.30
Ethnicity			**0.97**			**0.93**
Creole	1			1		
Qom	0.94 (0.26 - 3.37)	0.93		0.46 (0.25-0.87)	**0.02**	
Age x Ethnicity			**0.67**			0.07
Age x Creole	1			1		
Age x Qom	0.95 (0.90 – 1.00)	**0.06**		0.99 (0.94 - 1.04)	0.74	
Gender			0.29			0.25
Female	1			1		
Male	1.27 (0.84 - 1.92)	0.53		0.89 (0.52 - 1.54)	0.68	
Presence of *T. infestans*			**0.85**			**0.87**
No	1			1		
Yes and not infected, or ND*	0.72 (0.45-1.17)	**0.19**		0.47 (0.26-0.84)	**0.01**	
Yes and infected	1.77 (0.93-3.35)	**0.08**		0.19 (0.02-1.48)	**0.11**	
Parasite load				1.80 (1.48 – 2.20)	**<0.001**	**1**

*This category combines infested houses with uninfected bugs or infested houses with no data on infection status of the collected bugs.

Table 5: Top models selected by multimodel analysis for the infectiousness to the vector

	Model 1			Model 2
	1245	12345	125	256
logLik	-187.96	-187.30	-189.87	-103.55
AICc	388.68	389.63	390.28	218.04
ΔAIC	0	0.95	1.60	0
Model weight	0.34	0.21	0.15	0.45
1) Age	X	x	x	
2) Ethnicity	X	x	x	X
3) Gender		x		
4) Age x Ethnic	X	x		
5) Presence of *T. infestans*	X	x	x	x
6) Parasite load				x

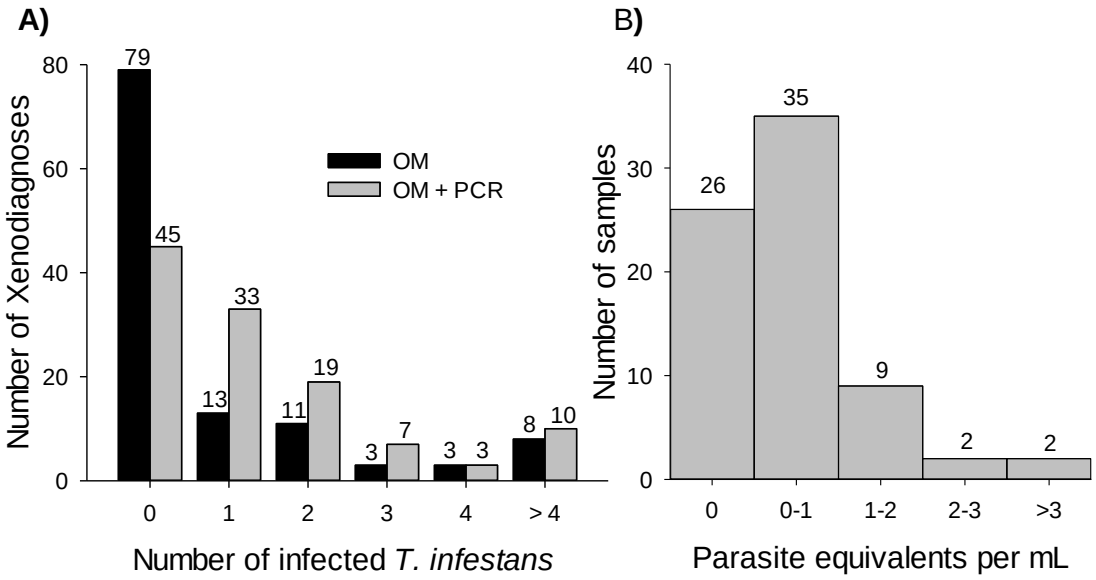

Figure 1: A) Distribution of *T. cruzi* infection in *T. infestans* employed in artificial xenodiagnosis using optical microscopy or kDNA-PCR diagnosis. B) Distribution of parasite load of infected inhabitants of Pampa del Indio.

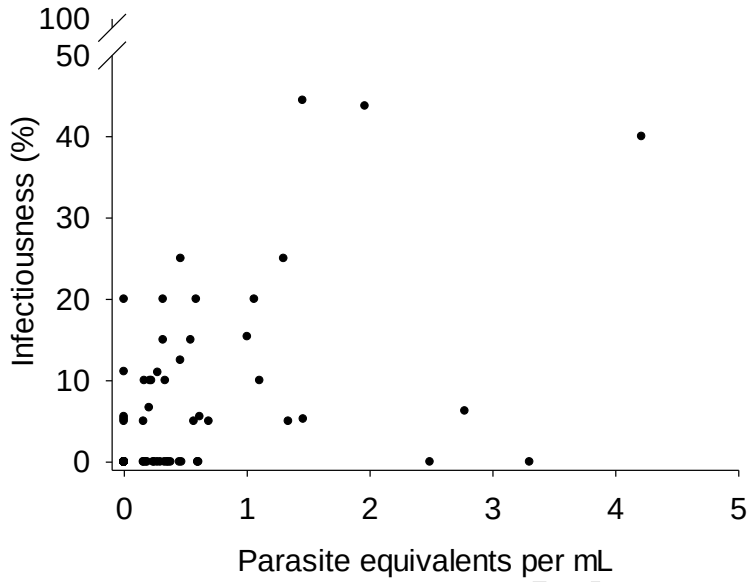

Figure 2: Distribution of infectiousness to *T. infestans* of *T. cruzi*-infected humans according to parasite load quantified by qPCR.

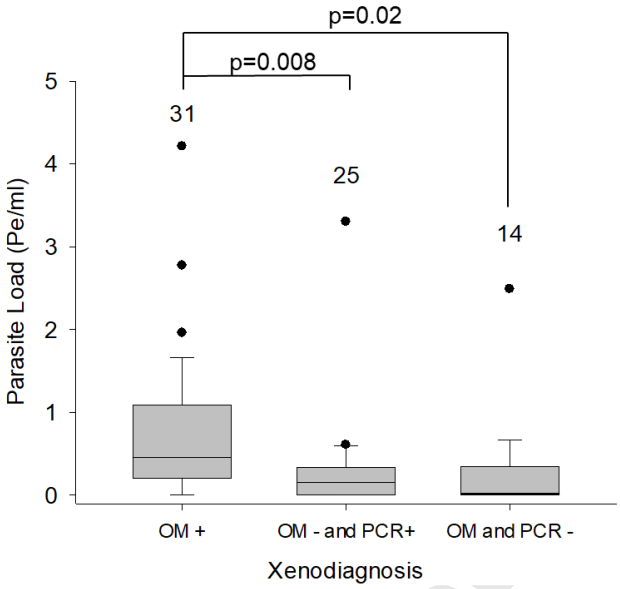

Figure 3: Parasite load distribution for XD results. Boxes include 1st and 3rd quartiles, lines show the mean, whiskers are the 95% confidence interval, dots represent outliers and the numbers above are the sample size for each XD result. P values are the significant values obtained from pairwise Wilcoxon tests.

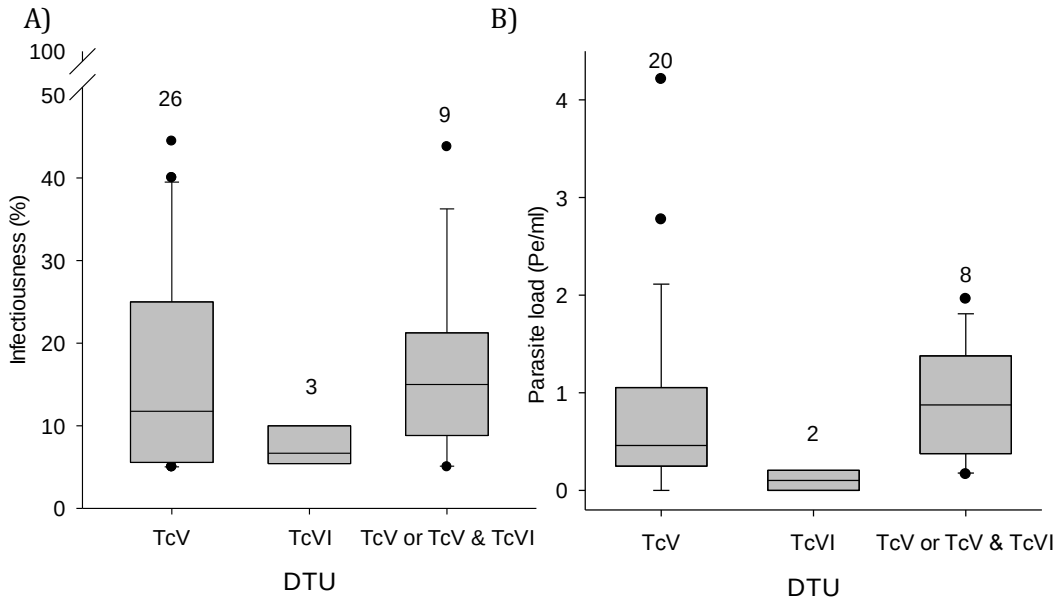

Figure 4: A) Distribution of human infectiousness and B) parasite load for the identified DTUs. Boxes include 1st and 3rd quartiles, lines show the mean, whiskers are the 95% confidence interval, dots represent outliers and the numbers above are the sample size for each DTU.

Highlights

- Seropositive residents of Pampa del Indio exhibited low infectiousness to the vector
- Infectiousness decreased with age and BMI.
- Exposure to *T. infestans* was negatively associated with infectiousness
- *T. cruzi* parasite load measured by qPCR was very low (<5 PE/ ml)

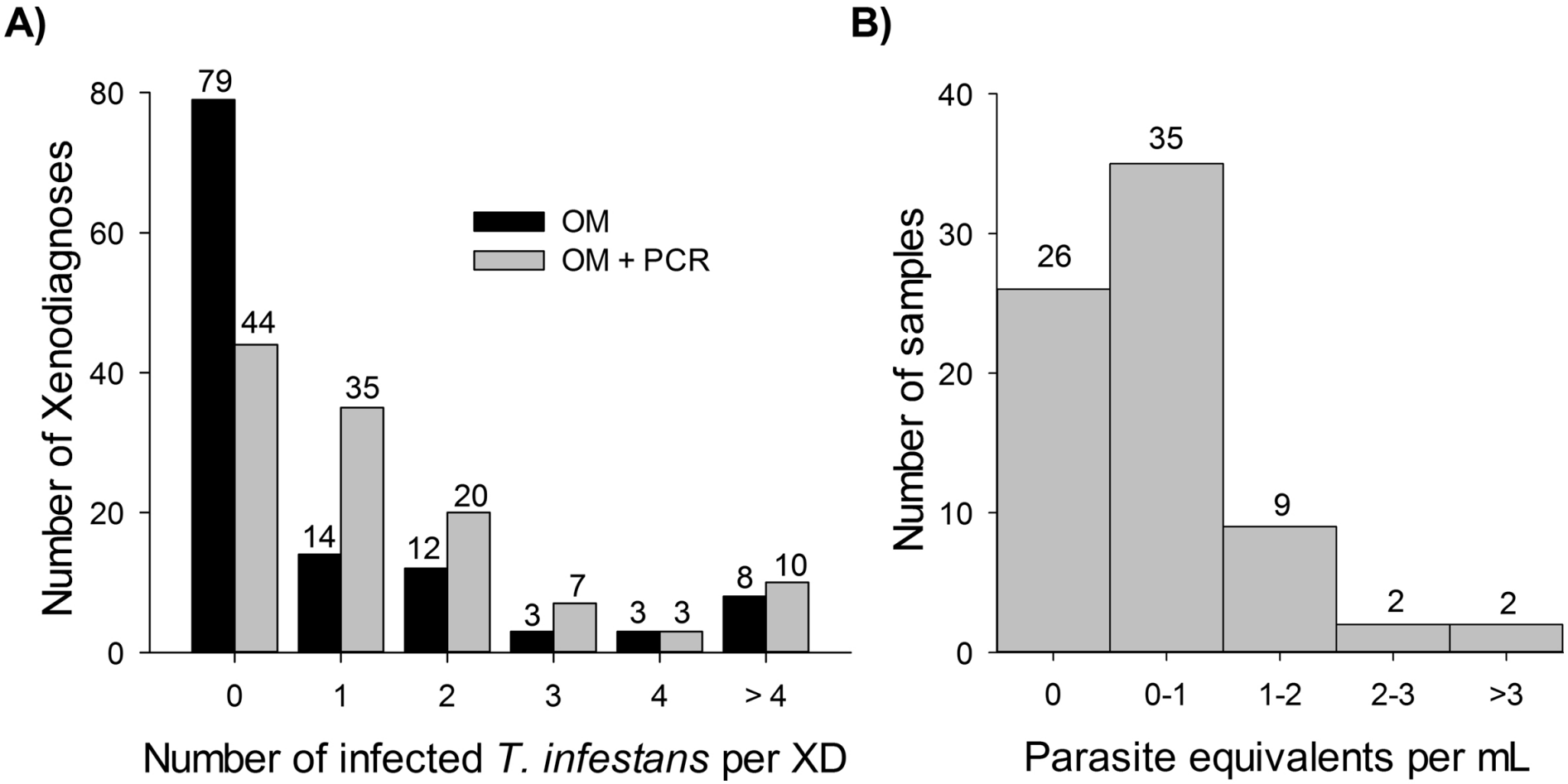
A)
B)
Number of Xenodiagnoses
80
60
40
20
0
79
44
14
35
12
20
3
7
3
3
8
10
OM
OM + PCR
0
1
2
3
4
> 4
Number of infected T. infestans per XD
Number of samples
40
30
20
10
0
26
35
9
2
2
0
0-1
1-2
2-3
>3
Parasite equivalents per mL

Figure 1

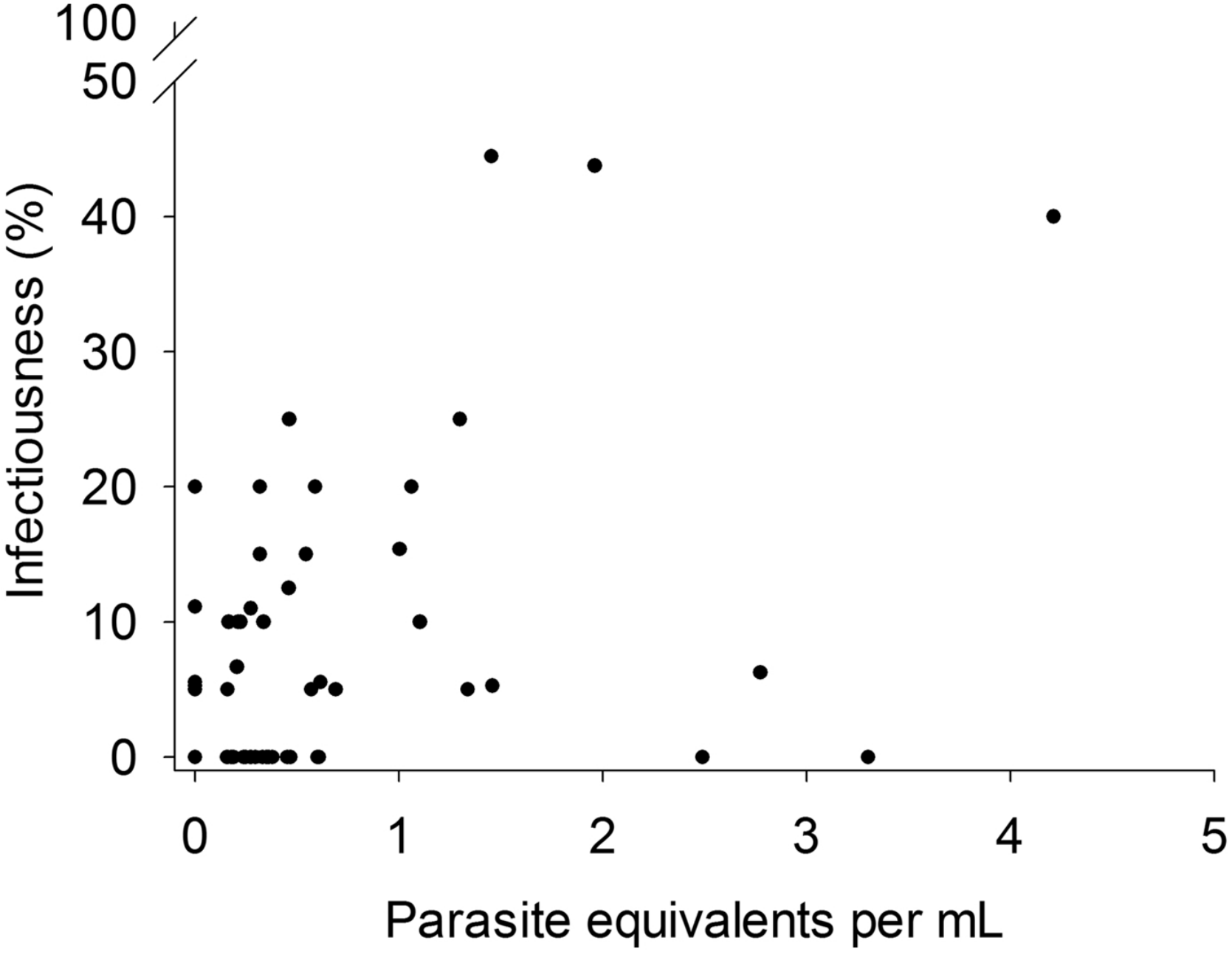
100
50
40
30
20
10
0
Infectiousness (%)
0
1
2
3
4
5
Parasite equivalents per mL

Figure 2

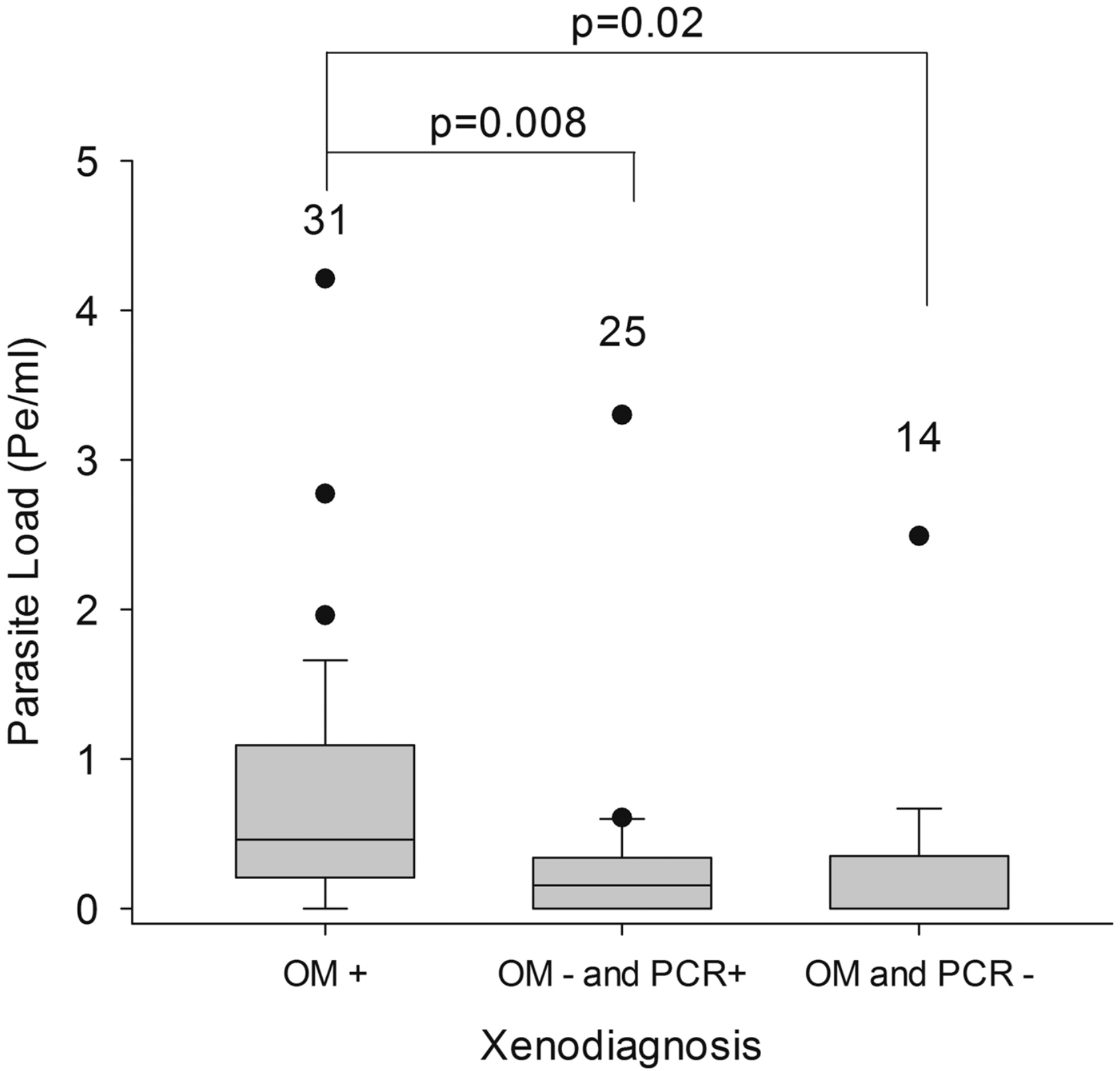
p=0.02
p=0.008
5
4
3
2
1
0
31
25
14
Parasite Load (Pe/ml)
OM +
OM - and PCR+
OM and PCR -
Xenodiagnosis

Figure 3

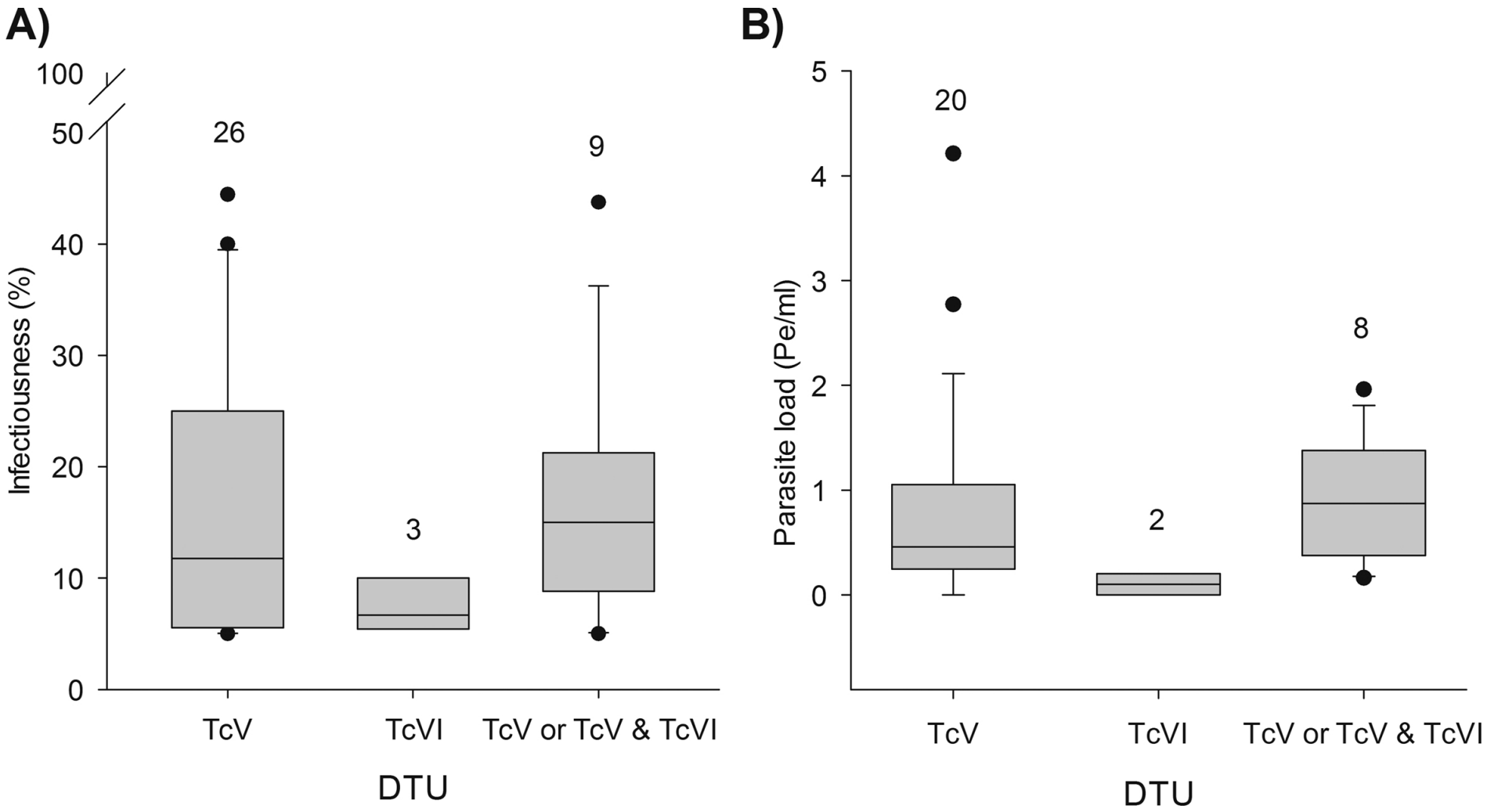
A)
100
50
40
30
20
10
0
Infectiousness (%)
26
3
9
TcV
TcVI
TcV or TcV & TcVI
DTU
B)
5
4
3
2
1
0
Parasite load (Pe/ml)
20
2
8
TcV
TcVI
TcV or TcV & TcVI
DTU

Figure 4