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Prevalence and distribution of parasites and pathogens of Triatominae from Argentina, with emphasis on *Triatoma infestans* and Triatoma virus TrV

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ABSTRACT

Chagas' disease is the most important endemic arthropod–zoonosis in Argentina with an estimated 1.6 million people infected with the causative agent *Trypanosoma cruzi*. *Triatoma infestans* is the main vector of Chagas' disease in Argentina. A survey for parasites and pathogens of Triatominae was conducted from August 2002 to February 2005. Collections of insects were made in domiciles, peridomiciles, and in the natural habitats of the Triatominae. Insects from these collections were dissected and their organs and tissues examined for flagellates. Frass from these insects was collected and examined for detection of the entomopathogenic virus Triatoma virus (TrV) using AC-ELISA and PCR. Triatominae belonging to four species, *T. infestans* ($n = 1646$), *Triatoma guasayana* ($n = 4$), *Triatoma platensis* ($n = 1$) and *Triatoma sordida* ($n = 5$) were collected from 62 sites located in 13 provinces of Argentina. Triatoma virus and two protozoan species, *Blastocrithidia triatomae* and *T. cruzi*, the etiological agent of Chagas disease, were found infecting Triatominae. The total prevalence of TrV in 1646 *T. infestans* analyzed by ELISA was 9.66% (159/1646) from 7 to 13 provinces where collections were made. Triatoma virus positive triatomines were found in 17 of 62 populations when examined by AC-ELISA but in 38 of 62 populations when PCR was used for detection. The prevalence of *B. triatomae* in *T. infestans* was 0.43% (7/1646), while the prevalence of *T. cruzi* was 1.3% (21/1646). This is the first study on the diversity, distribution and prevalence of flagellated protozoa and TrV of Triatominae in endemic Chagas' disease regions of Argentina.

1. Introduction

Chagas' is an endemic zoonotic disease in the America's that affects almost 12 million people (OPS, 2006). It is estimated that 1.6 million Argentineans are infected and approximately 15–30% of the cases results in cardiac damage or irreversible lesions in other organs (Segura et al., 2001). Triatomines constitute a very important link in the transmission of *Trypanosoma cruzi* (Chagas, 1909), the etiological agent of Chagas' diseases while *Triatoma infestans* (Klug) is the main vector species of Chagas' diseases in Argentina. Historically, control programs of Triatominae in Argentina have been conducted almost exclusively using synthetic insecticides. Continuous applications of chemical methods for vector control has often been associated with high levels of insecticide resistance and environmental and health concerns. Because of these concerns, alternative vector control is playing an increasingly important role in integrated management strategies.

More than 60 species of Triatominae have been found in nature to be affected by various natural enemies including predators, parasitoids, ecto and endoparasites and pathogens, as well as other poorly defined symbiotic associations (Ryckman and Blankenship, 1984; Weiser, 1991; Coscarón et al., 1999). Parasites and pathogens of Triatominae in Argentina are perhaps the most poorly known of these natural enemies.

Triatoma virus (TrV) is the only entomopathogenic virus found in Triatominae (Marti, 2005). Triatoma virus was identified by Muscio (1988) in *T. infestans* from Córdoba province, Argentina. Subsequently, biochemical, molecular, and crystallographic characterization was reported as well as genomic sequencing (Muscio et al., 1988; Czibener et al., 2000; Rozas-Dennis et al., 2004; Estrozi et al., 2008). This virus is composed of 35% single strand RNA and approximately 10,000 nucleotides and 65% protein. The viral particles are spherical with a diameter of 30 nm and lack an envelope.

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Triatoma virus was included in the Dicistroviridae family, a small group of viruses from insects, with the type species being the cricket paralysis virus (CrPV) (ICTVdB, 2002). Triatoma virus replicates in the midgut epithelium cells of triatomines, causing death of the infected insects (Muscio, 1988). Pathogenicity of the virus and the mechanisms of transmission for TrV have only partially been determined indicating that coprophagy is one of the main routes of infection in laboratory colonies (Muscio et al., 1997, 2000). Vertical transmission of TrV was suspected but not verified (Muscio et al., 1997). There is no information on the distribution or prevalence of TrV in natural populations of triatomines in Argentina.

Several species of protozoa in the genera *Blastocrithidia, Crithidia, Eimeria, Haemogregarina, Hepatozoon, Machadoella, Octosporea, Toxoplasma* and *Trypanosoma* have been found in triatomines, (Ryckman and Blankenship, 1984). The most virulent agents in triatomines are, *Trypanosoma rangeli* (Watkins, 1971; Zeledón and De Monge, 1966) and *Blastocrithidia triatomae* (Schaub, 1988a,b) the later being the Triatominae parasite most intensively studied. *B. triatomae* was described by Cerisola et al. (1971) in *T. infestans* from Córdoba province, Argentina, and different authors studied its ultrastructure (Mehlhorn et al., 1979), mode of transmission (Schaub et al., 1989), pathogenesis (Schaub, 1990a,b), encystment (Reduth and Schaub, 1988) and its culture in vitro (Reduth et al., 1989). In previous studies (Schaub, 1991) mortality rates of 30% were recorded in Triatominae infected with *B. triatomae*. Schaub (1990a) observed clear differences at the cuticle level between infected and healthy *T. infestans*.

Two fungal entomopathogens, *Beauveria bassiana* (Bals) Vuillemin (Marti et al., 2005) and *Paecilomyces lilacinus* (Thom) Samson (Marti et al., 2006) have previously been found infecting Triatominae in Argentina.

The aim of this study was to use classical and modern detection methods to determine the parasites and pathogens (including TrV) of Triatominae in different regions of Argentina. We collected 62 samples during a three years period in order to improve our knowledge on the diversity, prevalence and distribution of flagellated protozoa and TrV of Triatominae in Argentina.

2. Materials and methods

Triatominae were collected from 13 provinces of Argentina (Catamarca, Chaco, Córdoba, La Rioja, Mendoza, Misiones, Neuquén, Salta, San Juan, San Luis, Santa Fe, Santiago del Estero, and Tucumán) from August 2002 to February 2005. Random surveys of Triatoma population were made mainly during the spring and summer months when the greatest increases in the Triatominae species populations occurred. Field collections were made in domiciles, peridomiciles, and a few natural environments. Houses sampled in this study typically had adobe walls, a thatched roof, and two to four rooms or were houses with brick walls and roofs of corrugated metal sheeting. Such a dwelling covered by a single roof defined the domiciliary area. The peridomestic environment consisted of places located within the area of human activity as was defined by Bos (1988) and included a wide array of structures such as sheep, goat, and pig corrals, chicken coops and store rooms. In the wild environment, Triatominae were collected from bird nests and under the bark of trees located more than 300 m from the closest domiciles.

Triatominae were collected individually using metallic forceps; on some occasions in order to facilitate collection of the insects, dislodging substances such as tetramethrin 0.2% were used. Collected insects were transported individually to the laboratory in sterile plastic containers with folded pieces of paper inside and capped with a fine screen. Captured Triatominae were identified according to Lane and Wygodzinsky (1979) and maintained at a temperature of 27 ± 1 °C, 60% ± 5% relative humidity and a photoperiod of 12:12 h (light:dark).

Adults and nymphs (III, IV and V) were observed with a stereomicroscope in order to detect signs and symptoms indicating the presence of pathogens. Symptomatic and asymptomatic insects were dissected as described by Marti (2005) and their organs and tissues examined in fresh wet mounts and Giemsa (10%) stained smears and observed for protozoan infections. Frass samples from each insect were individually placed in Eppendorf tubes and dissolved in 200 µl of phosphate buffed saline (PBS) and kept at −70 °C until used for TrV detection. Detection of TrV infection in frass samples was performed with an antigen-capture enzyme-linked immunosorbent assay (AC-ELISA) and reverse transcription-polymerase chain reaction (RT-PCR) as described by Marti et al. (2008). Briefly, frass samples resuspended in PBS were homogenized in TRIZOL reagent (GIBCO-Invitrogen, USA), and vRNA was purified according to the manufacturer's instructions. For AC-ELISA, hen and rabbit TrV antisera produced in our laboratory were used as capture and detector antibodies, respectively. During 2004 and 2005 all samples were analyzed by AC-ELISA, afterwards, three samples at random from each collection were taken and processed by RT-PCR.

3. Results

A total of 1646 *T. infestans* and 10 insects of three other species of Triatominae, *Triatoma sordida* (Stäl) ($n = 5$), *Triatoma guasayana* (Wygodzinsky and Abalos) ($n = 4$) and *Triatoma platensis* (Neiva) ($n = 1$), were isolated from 62 separate collections made in domiciles (17.4%), peridomiciles (82%), and a few wild environments (0.6%). The RNA virus, Triatoma virus (TrV), and two flagellated protozoans, *B. triatomae* and *T. cruzi* were identified in the Triatominae species examined.

3.1. Triatoma virus

TrV positive tritomines by ELISA were detected in 17 of 62 populations. The range of TrV positive populations varied between 4.54% and 25%, with a single positive specimen of *T. sordida* captured in a house from the Chaco province. TrV infected individuals were found in seven provinces with the greatest levels found in La Rioja province where 5 of 17 populations were positive for TrV (Table 1). When considering the total number of insects examined

Table 1
Natural prevalence of Triatoma virus in Triatominae of Argentina by ELISA.

Province (city)	Date	Habitat	Prevalence (*n*)
S. del Estero (Jimenez)	December 2003	P	18.18% (4/22)
S. del Estero (Mayusca)	December 2002	D	13.33% (6/45)
San Luis (El Zampal)	April 2003	P	22.82% (76/333)
San Luis (Lomas)	April 2003	P	8.69% (2/23)
Mendoza (San Gabriel)	April 2003	P	9.65% (11/114)
Mendoza (Los Yauyines)	April 2003	P	4.54% (1/22)
Tucumán (Yangallo)	June 2003	P	10% (1/10)
Tucumán (Simoca)	March 2003	P	20% (7/35)
Tucumán (Leales)	December 2002	D	9.52% (2/21)
Chaco (Gral. Guemez)	October 2003	D	100% (1/1[a])
La Rioja (Cuatro Esquinas)	February 2004	P	16.11% (29/180)
La Rioja (La Lomita)	February 2004	P	13.04% (3/23)
La Rioja (Anillaco)	April 2004	P	5.77% (3/52)
La Rioja (Los molinos)	April 2004	P	25% (1/8)
La Rioja (San Blas)	April 2004	P	10% (3/30)
Santa Fe (Los Nocheros)	November 2004	P	11.94% (8/67)
Santa Fe (Villa Mineti)	November 2004	D	25% (1/4)

P = peridomicile, D = domicile.
[a] *Triatoma sordida*, all other triatomines were *T. infestans*.