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Detection of triatomine infection by Triatoma virus and horizontal transmission: Protecting insectaries and prospects for biological control

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ABSTRACT

Triatoma virus (TrV) is the only triatomine entomopathogenic virus identified so far. Propagation of TrV in insectaries depends on handling procedures and triatomine population dynamics. The effects of propagation can be devastating and entire colonies must often be sacrificed to prevent spread of the virus throughout the insectary. This study found that after 41.3 days from TrV ingestion of human blood with 0.04 mg of viral protein by 5th instar *Triatoma infestans*, viral particles could be detected by RT-PCR; in a second horizontal transmission experiment time to detection resulted in a mean of 42.5 days. These results should rise awareness of TrV dynamics in nature, help estimate the spread of this virus when TrV-infected field-collected insects are incorporated into an insectary, and provide a base for the consideration of TrV as an agent of biological control of some species of triatomines.

1. Introduction

Chagas' disease is an endemic zoonosis in the American continent that affects around 7–8 million people (WHO, 2013). Triatomines constitute the dominant source of infection in the transmission of *Trypanosoma cruzi* (the etiological agent of Chagas' disease), and *Triatoma infestans* is the main vectorial insect species in Argentina, Bolivia and Paraguay. Approximately 70 species of triatomines have been found to be affected in nature by various natural enemies including predators, parasitoids, ecto- and endoparasites, and pathogens (Marti, 2010). Triatoma virus (TrV) is the only entomopathogenic virus of triatomines identified to date; it replicates within the cytoplasm of cells of the digestive tract of triatomines (Muscio et al., 1988). It was first found in *T. infestans* in domiciliary and peridomiciliary habitats in Northern Argentina (Muscio et al., 1987; Marti et al., 2009), but TrV also infects the sylvatic species *Psammolestes coreodes*, and *T. delpontei* in the provinces of northwestern Argentina (Susevich et al., 2012). TrV was detected in insectaries in Argentina, causing over 90% mortality in *T. infestans* and to date, there has been no evidence of TrV infection in humans (Muscio et al., 2000) or mice (Querido et al., 2013).

Recently TrV was recorded in four triatomine species (*Rhodnius neglectus*, *R. prolixus*, *T. infestans* and *Meccus longipennis*) from an insectary in Brazil and in *T. infestans* from two insectaries in Argentina (Marti et al., 2013). Horizontal transmission of TrV in triatomines through cannibalism and coprophagy has been demonstrated, producing high mortality, delayed development, and reduced fecundity in infected insects (Muscio et al., 1997, 1988). The propagation of TrV in insectaries depends on insectary handling procedures and host population dynamics, and its effects on mortality can be devastating, with colonies often having to be sacrificed to prevent the spread of the virus to the entire insectary (Marti, unpublished result).

Triatomines defecate during or immediately after feeding, and their feces may be found in the insectaries' rearing containers. As TrV remains infective in the feces, healthy insects may become infected by coprophagy (also called cleptohaematophagy), which is a common behavior in triatomines (Schaub et al., 1989).

The goal of this work was to determine the time between ingestion of the viral TrV particles to the detection by reverse-transcription polymerase chain reaction (RT-PCR) in fecal matter of *T. infestans*, to estimate the rate of horizontal transmission, in order to increase our knowledge of the dynamics of TrV once it

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enters an insectary, and to verify that field insects incorporated into an insectary are TrV free. This information will also improve our knowledge in order to assess the potentiality of TrV as a biological control agent of *T. infestans* populations.

2. Materials and methods

2.1. Time to TrV detection

We used 20 nymphs 5th stage of *T. infestans* recently molted and verified TrV-free by RT-PCR analysis (Marti et al., 2008). The insects were weighed and fed individually (10 days after molting into the 5th stage) during 1 h using an artificial feeder with human blood, free of infection (*Trypanosoma cruzi*, HIV, HTLV, Brucellosis, Hepatitis and Syphilis), and containing Adenin–Dextrose–Phosphato–Citrate (ADPC) as anticoagulant; the blood was obtained from the "Instituto de Hemoterapia" in the city of La Plata, Argentina. The artificial feeder used in our experiments was designed in our laboratory, after a modification from Aldana et al. (2005) (Fig. 1). TrV was added to the blood in the feeder as a solution of viral proteins of 0.2 mg/ml, to reach a final concentration of 0.04 mg of protein. To allow for the ejection of the initial diuresis that takes place during or immediately after feeding, within two hours after feeding the 20 nymphs were weighed again, to calculate the weight increase of each individual; the latter provided the net amount of blood ingested (microliters), which in turn provided an estimation of the amount of viral proteins ingested (in micrograms) by each insect.

After feeding with the infected blood, each insect was placed individually in 170 cm^3 plastic containers, and thereafter fed for 30 min every 15 days using the same artificial feeder, but with non-infected blood; the containers had a film paper on the bottom, which was removed daily to obtain the feces excreted naturally by the insects (i.e., no abdomen compression was used to avoid causing possible injuries). The detection of TrV infection was performed using samples of dried feces resuspended in phosphate buffered saline (PBS), which were analyzed by RT-PCR (Marti et al., 2008). To assess the sensitivity of the RT-PCR procedure, RNA was extracted from the 0.2 mg/ml TrV solution that was used as a positive control for all the diagnostic tests performed. The protein concentration of the selected samples was determined by the Bradford total protein content assay using a Bio-Rad Protein Assay Kit (Bio-Rad) with bovine serum albumin (BSA) as the standard. The analytical sensitivity of the test was determined using 10-fold serial dilutions of the cDNA obtained from original purified virus containing 0.01 mg of viral proteins. The minimal concentration of RT-PCR was found to be 0.001 ng of viral proteins.

Linear correlations and analysis of covariance (ANCOVA) were carried out; the latter verifies all possible linear combinations of sources of random variation and is used to estimate the error terms for testing statistical significance of effects. These analyses allowed us to assess the possible relationship between the amount of viral proteins ingested by each insect and both the time to detection in their feces and the time to its death, by sex we used the software Statistica (StatSoft, 2009).

2.2. Rate of horizontal transmission

To measure the rate of TrV horizontal transmission 10 recently-emerged TrV-free adults (analyzed by RT-PCR) were placed in 400 cm^3 plastic containers, with one male and one female, both infected with TrV; all insects were individually marked, and this set-up was replicated three times. After 25 days, the feces from each individual were collected by abdominal compression, and this

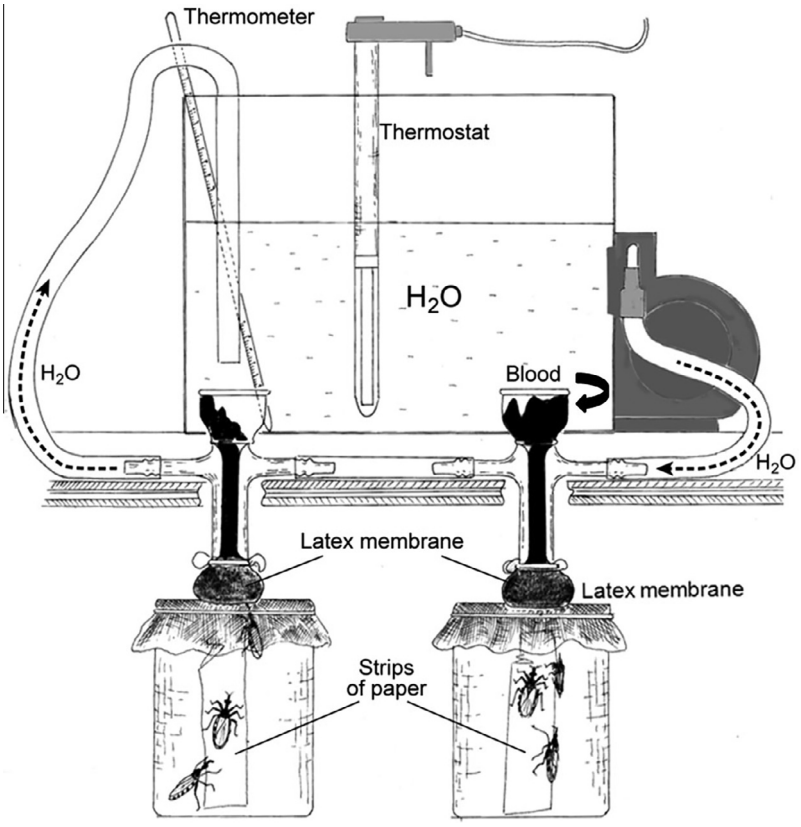

Fig. 1. The artificial feeder it basic structure consists of two glass vials containing a latex membrane with human blood kept at 37 °C by water maintained at that temperature by an electric thermostat as a heat source.