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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.

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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

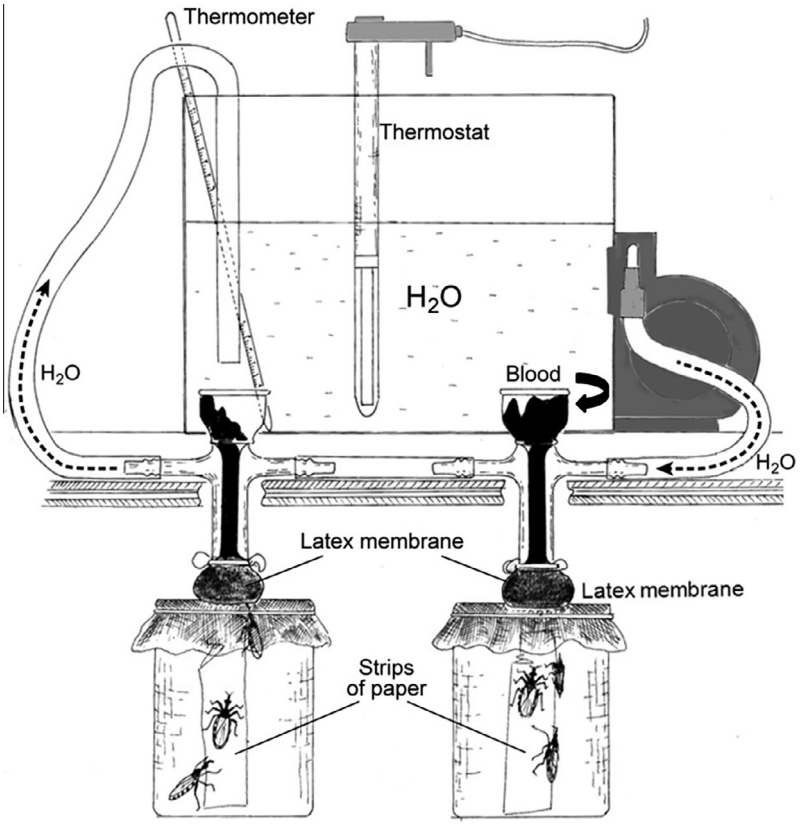

Fig. 1. The artificial feeder it basic structure consists of two glass vials containing a latex membrane with human blood kept at 37 °C by water maintained at that temperature by an electric thermostat as a heat source.

procedure was repeated every 15 days until all the insects had died; until the end of the experiment the insects were fed with TrV-free human blood using an artificial feeder for 30 min every 15 days. The fecal samples of each individual was resuspended in 200 μL of PBS and analyzed by RT-PCR.

3. Results

3.1. Time to TrV detection

The mean weight of the 20 5th stage nymphs of *T. infestans* before being fed was 0.079 g (±0.025 SD). The mean weight of TrV proteins ingested was 0.036 ± 0.026 μg, and 17 (85%) of the 20 individuals tested resulted positive. The presence of TrV particles in feces was detected within a mean (±std. dev.) period of 41.4 ± 16.4 days (range = 15–70 days) since infection, with no significant relationship of the detection time as a function of the amount of TrV particles ingested (Fig. 2A). We found that a simple linear regression between the amount of TrV particles ingested and time of death of the insects since ingestion was statistically significant ($y = 250.2397 - 1849.911 * x$; $r = -0.5589$, $p = 0.0104$), however, the regression between the amount of TrV particles ingested and time of death of the insects since confirmed positivity in feces was not statistically significant ($y = 215.8718 - 1795.0559 * x$; $r = -0.4798$, $p = 0.0513$) (Fig. 2B).

The same linear regressions were checked separately by sex, and a comparison with analysis of covariance (ANCOVA) of the slopes of the effect of the amount of TrV ingested on the time from first positivity test in feces until death, resulted in differences not statistically significant between sexes (results not shown).

3.2. Rate of transmission

Only one individual out of 30 (96.6%) was not positive to TrV and died, possibly due to abdominal compression. The mean detection time (±std. dev.) was 42.5 ± 18.1 days (range = 25–85 days).

4. Discussion

Although the mean time between infection and detection was estimated in 41.3 days, this is probably an underestimation, as we could not establish the exact date of detection because there were intervals of up to nine days between the last negative and the first positive samples. Contrary to the statistically significant regression results between the amount of TrV particles ingested and time of death of the insects since ingestion, the regression between the amount of TrV particles ingested and time of death of the insects since confirmed positivity in feces was not significant; we believe that this difference can be explained by the added variability in the time between infection and detection (that showed a 40% coefficient of variation, with a range of 15–70 days).

The possibility that viral particles may have gone through the digestive tract without causing infection can be dismissed because in *R. prolixus*, total loss of the weight of ingested blood was obtained 20 days after ingestion (Schilman and Lazzari, 2004) and for *T. infestans* the human blood was detected by PCR in 100% of the insects after 14 days post feeding (Pinto et al., 2012). Additionally, it is known that the virus undergoes an environmental pH variation along the digestive tract during the infection process, resulting in a destruction of the TrV capsid, and the release of the viral genome and reassembly (Snijder et al., 2013); as in our experiments TrV was detected in only one individual before 20 days, we may dismiss the possibility that the viral particles traversed the entire digestive tract without producing infection.

The two experiments we carried out were extremely similar in terms of time between infection and detection (41.4 ± 16.4 days in the first experiment (see Section 2.1) on time to detect infection using blood feeder, and 42.5 ± 18.1 days in the second experiment (see Section 2.2) on the rate of horizontal transmission by coprophagy). We believe that this similarity could be explained by the fact that triatomines need to ingest digestive symbionts by ingesting the feces (coprophagy) (Schaub et al., 1989), compelling the insects to be continuously in contact with their feces; thus the introduced TrV-infected adults quickly generated a source of TrV particles while defecating, explaining such a rapid transmission with other healthy insects (second experiment). The efficacy of this process resulted in a successful infection of 96.6% of healthy insects in a time period of about 42.5 ± 18.1 days; of course this result may be associated to the particular ratio of healthy (10 individuals) to initially infected (2 individuals); despite this is a relatively reasonable ratio (≈17% of infected individuals in each container's population as a whole) new experiments with lower ratios should be carried out to verify the sensitivity of the horizontal transmission to the ratio infected/non-infected population.

Life history parameters are frequently estimated for many species of triatomines (Medone et al., 2012) and one of their characteristics is its high variability, normally attributed to environmental factors (temperature, humidity, feeding source and feeding regime); however our results, and the fact that TrV has already been found in various insectaries of Argentina and Brazil, suggest that part of that variability in some population parameters in triatomine species may also be affected by the presence of TrV. The results from our experiments may be used as diagnostic to test if triatomine colonies within an insectary are Trv-free.

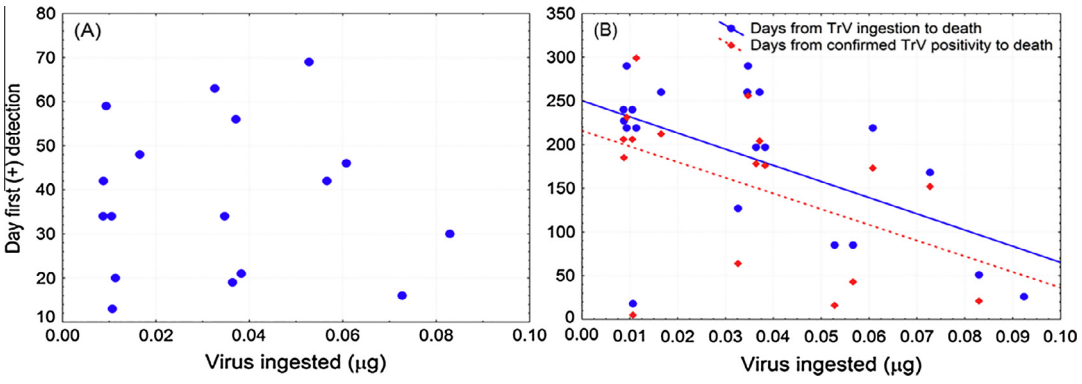

Fig. 2. Relationship between the TrV particle load and the time of detection of positivity in feces and the amount of TrV particles ingested (μg) (Fig. 2A), and linear regressions between the time (days) until death after the ingestion of the TrV particles and until death after the first evidence of positivity to TrV in feces, both as a function of the amount of TrV particles ingested (μg) (Fig. 2B); the regressions were statistically significant (see text).

5. Conclusions

Our conclusion can be stated in the form of a recommendation: when field-collected individuals are added to the colonies of an insectary, they should be put under quarantine, and checked for infection 50–60 days after their incorporation; thanks to the high sensitivity of the PCR technique this can be done using a sample of pooled feces from the container where they are held, so as to confirm that this virus is not present and thus ensure good health of all colonies. Additionally, the success shown by a 96.6% of horizontal infection suggests that the TrV is a promising biological control agent of *T. infestans* populations.

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