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Short Communication

New Triatoma virus hosts in wild habitats of Argentina

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ABSTRACT

Triatoma virus (TrV), a member of the *Dicistroviridae* family, replicates in intestinal epithelial cells, causing delayed development and death of infected individuals. The aims of this study were to find naturally infected species of Triatominae in the wild in the region endemic for Chagas disease and analyze and compare the sequence diversity of TrV obtained from different Triatominae. A total of 253 Triatominae belonging to 10 species were captured by active or passive collection. Three new sequences were obtained from *Triatoma infestans, Triatoma delpontei* and *Psammolestes coreodes* and the analysis revealed that these sequences were very similar. *Ps. coreodes* is a new host for TrV.

1. Introduction

The Triatoma virus (TrV) is a viral pathogen of *Triatoma infestans* first identified by Muscio in 1988 in Córdoba Province, Argentina (Muscio et al., 1988). TrV is a member of the *Dicistroviridae* family, a small group of viruses from insects whose type species is the cricket paralysis virus (ICTVdB, 2002). TrV is transmitted both vertically (transovarial) and horizontally (cannibalism and coprophagy) (Muscio et al., 2000). The infection causes developmental delays, reduced oviposition and premature death (Rozas-Dennis and Cazzaniga, 2000; Muscio et al., 1997). Due to its vertical transmission and high pathogenicity, TrV is considered a potential agent for biological control of *T. infestans* (Muscio et al., 1997), the vector of the protozoan parasite *Trypanosoma cruzi*, which causes Chagas disease in an estimated 8–11 million people in Latin America. For that reason, TrV could potentially be exploited to manage its pestiferous hosts (Gordon and Waterhouse, 2006). Susceptibility of the species is determined by the entry route: ingestion, intrahemocelic infection or natural occurrence in the field (Marti et al., 2009). Nine species (*T. infestans, T. platensis, T. delpontei, T. pallidepennis, T. rubrovaria, T. guasayana, T. patagonica, T. sordida* and *Rhodnius prolixus*) are known to be susceptible to TrV (Marti et al., 2009). The aims of this study were to find other naturally infected species of Triatominae in the wild microhabitat of the Provinces of Chaco and La Rioja, Argentina, and then to analyze and compare the nucleotide sequence diversity of TrV obtained from different Triatominae in order to reveal the level of diversity among the species present in Argentina.

Triatomines were collected during eight trips of approximately 10 days each, in small cities endemic for Chagas disease in the provinces of Chaco and La Rioja (Gran Chaco region) during 2006–2011. Triatomines were captured strictly in the wild, in different microhabitats such as bird nests, mammal refuges, cacti, bromeliads and tree bark (Brewer et al., 1978; Wisnivesky-Colli et al., 1997), both by active and passive collection. This information (i.e. microhabitat where the insect was collected) was carefully recorded. For active collection, triatomines were collected individually using metallic forceps and, on some occasions, using dislodging substances such as tetramethrin 0.2% to facilitate collection of the insects. For passive collection, Noireau traps were left overnight in each microhabitat with a mouse as bait in an external adhesive mesh, to capture the Triatominae (Abad-Franch et al., 2000). In addition, eight light traps were placed every 100 meters (a transect of about 800 m) and left during 4 h, at sunset, six nights per trip. Samples were geo-located using a handheld Garmin™ legend GPS navigator. The insects collected were transported individually to the laboratory in sterile plastic containers, each of which also contained a folded piece of paper and was capped with a fine mesh screen and then identified according to Lent and Wygodzinsky (1979), and maintained at a temperature of 28 ± 1 °C, 60% ± 5% relative humidity and a photoperiod of 12:12 h (light:dark). Approximately 10 mg of fecal samples from the triatomines was homogenized with 200 μl of phosphate buffer (PBS) and then stored in microtubes at −20 °C; RNA was extracted from these fecal samples. A volume of 50 μl of samples resuspended in PBS was homogenized in 300 μl TRIZOL reagent (GIBCO-Invitrogen,

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USA), according to the manufacturer's instructions. RNA concentration was determined by measuring absorbance at 260 nm (A260) in a spectrophotometer (A260 = 1 is equivalent to 40 lg/ml RNA). A ratio of A260/A280 = 2.0 was obtained for pure vRNA. RT-PCR was performed following the methodology of Marti et al. (2008). Primer sequences were selected from TrV-ORF2 regions that code for VP3 and VP1 capsid proteins, based on the GenBank AF 178440 sequence. The first TrV strain obtained from *T. infestans* captured in peridomiciliary environments in 2001 (Guanaco Muerto, Dean Funes, Córdoba Province) was used as positive control. These triatomines are maintained in the insectary of the Centro de Estudios Parasitológicos y de Vectores (CEPAVE), La Plata, Argentina. The PCR products were purified using Wizard SV Gel and PCR Clean-Up System (Promega, USA), cloned using a pGEM-T Easy Vector Systems (Promega, USA) and then used to transform competent Top 10 cells. The plasmid DNA was extracted and purified using the Wizard PlusMiniprep DNA Purification System (Promega, USA). Both strands were sequenced using M13 standard primers by Dye-Terminator SANGER. The sequences were edited using BioEdit software version 5 (Hall, 1999). Homology analyses were performed with the BLASTN program [http://www.ncbi.nlm.nih.gov/BLAST/]. The sequences were aligned in the MEGA program version 4.0 using the ClustalW algorithm. The sequence pair distances were calculated by DNAstar (Tamura et al., 2007). The phylogenetic trees were constructed using MEGA by the neighbor-joining (NJ) method and bootstrap analyses were conducted using 1000 replicates. Evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2007).

A total of 253 Triatominae belonging to 10 species were captured (Table 1). The presence of TrV was only detected in bird nests: *T. infestans* and *Psammolestes coreodes* in nests of Furnariidae ovenbirds (lark-like brushrunner) and *T. delpontei* in nests of Psittaciformes (parrots). The GPS locations were: S27°04′55.2″W61°31′54.5″ (*T. infestans*), S27°05′20.9″W61°31′59.4″ (*T. delpontei*) and S27°05′13.2″W61°31′51.5″ (*Ps. coreodes*). The three new sequences obtained by RT-PCR from *T. infestans*, *T. delpontei* and *Ps. coreodes* were named TIN2-ARG, TDE1-ARG and PCO1-ARG respectively. The nucleotide sequences obtained were submitted to GenBank under accession numbers HM044312, HM044314 and HM044315 (TDE1-ARG, TIN2-ARG and PCO1-ARG respectively). The sequences were compared to sequence AF178440 available in GenBank, the first strain of TrV isolated in Argentina, and to the sequence from a TrV-infected colony of *T. infestans* maintained at CEPAVE since 2001 and annotated in this study under accession number HM044313 (TIN1-ARG). Computer analysis showed very few differences with the TrV sequence reported in GenBank (Czibener et al., 2000) in nucleotides (positions 86, 222, 344, 374, 449 and 750) resulting after translation to putative 276 amino acid sequences in two changes (positions 74 and 250 – K or E and V or L respectively). No deletions or insertions were detected and only a few point mutations were observed. The highest level of nucleotide identity was found between PCO1-ARG, TIN2-ARG and AF178440 (100%). A phylogenetic analysis was performed on the amino acid sequences using the neighbor-joining tree method, and robustness of the results was assessed using 1000 bootstrap replicates. As shown in Fig. 1, two discrete clusters were formed: one with TIN2-ARG, AF178440 and PCO1-ARG and the other with TIN1-ARG and TDE1-ARG, demonstrating relatedness of amino acid sequence similarity, with a high bootstrap value.

Previous results have shown the presence of TrV in *T. infestans* only in homes and around houses (peridomiciliary environments) (Marti et al., 2009). However, in the present work, we found TrV in *T. infestans*, *T. delpontei* and *Ps. coreodes* in nests in the wild, with *Ps. coreodes* as a new host for TrV, thus increasing the number of susceptible species to ten. This could indicate that this virus is circulating naturally among species that have been observed in some cases co-inhabiting the same ecological niches. The refractory nature of birds to *T. cruzi* is well known (Teixeira et al., 2006). Birds could also be considered as refractory to TrV, as it occurs with *T. cruzi*, or they could develop an antibody response, if they have been infected with TrV. This possibility was not analyzed in the present work. Although birds might be considered as reservoirs of TrV, assuming that triatomines are feeding on birds, it would be highly uncertain to affirm this, because the triatomine colonies kept in our laboratory are fed on a hen, which has developed specific antibodies to TrV with no clinical signs (Marti, personal communication).

Since TrV was discovered in *T. infestans* (Muscio et al., 1988) and is geographically distributed in Argentina (Marti et al., 2009), its potential use as a biological control agent against the vectors of Chagas disease could be considered as a valid option. Nevertheless, the prevalence of TrV in triatomines in other Latin American endemic areas of Chagas disease is still unknown. The TrV dynamics reinforce the consideration of the possible use of this virus as a biological control agent because, at present, the programs for vector control of Chagas disease involve exclusively the use of chemicals in homes and around houses. To date, no test has been carried out in wild environments and these products have not been supplemented with any of the known biological agents. Elucidation of

Table 1
Number and distribution of Triatominae individuals captured in the wild.

Species	Method				Habitat	Prevalence (n)[b]	GPS[c] and province	Year of isolation
	Light[a]	Noireau[a]	Manually	Total				
Triatoma infestans[1]	24	1	5	30	**Bird nests** and palm tree	50% (1/2)	S27°08′14″W61°34′26.1″ (Chaco) S27°05′9.7″W61°31′43.3″ (Chaco)	2007
Triatoma sordida	0	10	12	22	Bird nests and tree bark		S25°28′1.1″W61°54′14.5″ (Chaco)	
Triatoma guasayana	13	0	17	30	Bird nests and dried cactus		S30°12′52.2″W67°35′36.7″ (La Rioja) S27°05′9.7″W61°31′43.3″ (Chaco)	
Triatoma delpontei[2]	0	0	30	30	**Bird nests**	100% (1/1)	S27°05′12.5″W61°32′28.1″ (Chaco)	2007
Triatoma eratyrusiforme	5	0	0	5			S30°12′52.2″W67°35′36.7″ (La Rioja)	
Triatoma garciabesi	20	0	10	30	Bird nests		S27°08′14″W61°34′26.1″ (Chaco) S30°11′63.8″W66°55′54″ (La Rioja)	
Psammolestes coreodes[3]	0	0	30	30	**Bird nests**	25% (1/4)	S27°04′43″W61°31′30.7″ (Chaco)	2007
Panstrongylus guentheri	16	0	0	16			S27°08′14″W61°34′26.1″ (Chaco) S27°05′9.7″W61°31′43.3″ (Chaco)	
Triatoma platensis	1	0	29	30	Bird nests		S27°05′14.1″W61°31′49.8″ (Chaco)	
Triatoma breyeri	0	30	0	30			S30°10′24.3″W66°57′25.1″ (La Rioja)	
Total				253				

The new sequences obtained in this study are: 1TIN2-ARG, [2]TDE1-ARG, [3]PCO1-ARG. The habitat where it was isolated is indicated in bold and underlined.
[a] The origin of the triatomines captured by traps is unknown.
[b] Prevalence of TrV and number of insects is indicated only in the three positive bird nests.
[c] Each GPS include a sampling area of approximately 400 Ha.

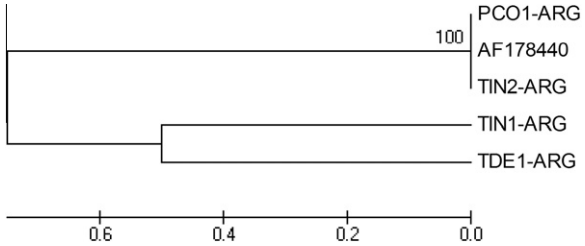

Fig. 1. Phylogenetic tree obtained by the neighbor-joining method from analysis of the putative amino acid of ORF 2 of Argentine and reference TrV sequences. The neighbor-joining tree was produced using MEGA program version 4.0 and the robustness of the tree tested using 1000 bootstrap replicates. Numbers above branches indicate support values.

their numbers and diversity, host, and pathological effects may reveal major roles for dicistroviruses in the biosphere (Bonning and Miller, 2010). The Triatominae colonies living in insectaries of Latin American countries periodically receive new insects from the field, which could be infected with TrV. This means that the free colony is under high risk of infection that could destroy insectaries. Taking this possibility into account, insectaries could be a source of TrV. This is one of the reasons why the presence of TrV in Latin American insectaries should be evaluated. Regarding the six mutations found at nucleotide level, they resulted in only two amino acid changes (position 74 K – basic- for E –acidic- and position 250 V or L, both neutral), with the former being complementary but tolerated. Viral capsid proteins have been shown to be suitable targets for phylogenetic studies in other dicistroviruses (Hunter et al., 2006; Tapaszti et al., 2009). De Miranda et al. (2004) showed that the isolates of Kashmir bee virus and acute bee paralysis virus can be broadly separated by their continent of origin, but it is more difficult to identify regional trends within each continent. Although we are aware that the number of analyzed sequences is low, no similar sequences are available either in Argentina or other parts of the world.

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References

Abad-Franch, F., Noireau, F., Paucar, A., Aguilar, M., Carpio, C., Racines, J., 2000. The use of live bait traps for the study of sylvatic *Rhodnius* populations (Hemiptera: Reduviidae) in palm trees. Trans. R. Soc. Trop. Med. Hyg. 94, 629–630.

Bonning, B.C., Miller, W.A., 2010. Dicistroviruses. Ann. Rev. Entomol. 55, 129–150.

Brewer, M.D., Argűello, N., Delfino, M., Gorla, D., 1978. Parasitismo natural de *Telenomus fariai* Costa Lima, 1927 (Hymenoptera, Scelionidae) en monte y presencia de Proanastatus excavatus De Sanis 1952 (Hymenoptera, Eupelmidae) parasitoide oófago de Triatominae en el departamento de Cruz del Eje, Córdoba. República Argent. Ann. Soc. Entomol. Brasil 7, 141–154.

Czibener, C., La Torre, J.L., Muscio, O.A., Ugalde, R.A., Scodeller, E.A., 2000. Nucleotide sequence analysis of Triatoma virus shows that it is a member of a novel group of insect RNA viruses. J. Gen. Virol. 81, 1149–1154.

De Miranda, J.R., Drebot, M., Tyler, S., Shen, M., Cameron, C.E., Stoltz, D.B., Camazine, S.M., 2004. Complete nucleotide sequence of Kashmir bee virus and comparison with acute bee paralysis virus. J. Gen. Virol. 85, 2263–2270.

Gordon, K.H.J., Waterhouse, P.M., 2006. Small RNA viruses of insects: expression in plants and RNA silencing. In: Bonning B.C. (Ed), Insect Viruses: Biotechnological Applications, San Diego, Elsevier, pp. 459–502.

Hall, T.A., 1999. BioEdit: a user friendly biological sequences alignment editor and analysis program for Window 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Hunter, W.B., Katsar, C.S., Chaparro, J.X., 2006. Molecular analysis of capsid protein of *Homalodisca coagulata* Virus-1, a new leafhopper-infecting virus from the glassy-winged sharpshooter, *Homalodisca coagulata*. J. Insect. Sci. 6, 1–10.

ICTVdB, 2002. Index of Viruses, <http://www.ncbi.nlm.nih.gov/ictvdb/Ictv/fs_dicis>.

Lent, H., Wygodzinsky, P., 1979. Revisión of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagaś Diseases. Bull. Am. Mus. Nat. Hist. 163, 123–520.

Marti, G.A., González, E.T., García, J.J., Viguera, A.R., Guérin, D.M.A., Echeverría, M.G., 2008. AC-ELISA and RT-PCR assays for diagnosis of triatoma virus (TrV) in triatomines (Hemiptera: Reduviidae) species. Arch. Virol. 153, 1427–1432.

Marti, G.A., Echeverría, M.G., Susevich, M.L., Becnel, J.J., García, J.J., 2009. Prevalence and distribution of some parasites and pathogens of Triatominae from Argentina, with emphasis on *Triatoma infestans* and Triatoma virus TrV. J. Invertebr. Pathol. 102, 233–237.

Muscio, O.A., La Torre, J.L., Scodeller, E.A., 1988. Characterization of Triatoma virus, a picorna-like virus isolated from the triatomine bug *Triatoma infestans*. J. Gen. Virol. 69, 2929–2934.

Muscio, O.A., La Torre, J.L., Bonder, M.A., Scodeller, E.A., 1997. Triatoma virus pathogenicity in laboratory colonies of *Triatoma infestans* (Hemiptera: Reduviidae). J. Med. Entomol. 34, 253–256.

Muscio, O.A., Bonder, M.A., La Torre, J.L., Scodeller, E.A., 2000. Horizontal transmission of Triatoma virus through the fecal–oral route in *Triatoma infestans* (Hemiptera: Triatomidae). J. Med. Entomol. 37, 271–275.

Rozas-Dennis, G.S., Cazzaniga, N.J., 2000. Effects of Triatoma virus (TrV) on the fecundity and moulting of *Triatoma infestans* (Hemiptera: Reduviidae). Ann. Trop. Med. Parasitol. 94, 633–641.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: molecular evolutionary genetic analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.

Tapaszti, Z., Forgárch, P., Kovago, C., Topolska, G., Nowotny, N., Rusvai, M., Bakonyi, T., 2009. Genetic analysis and phylogenetic comparison of Black queen cell virus genotypes. Vet. Microbiol. 139, 227–234.

Teixeira, A.R., Nascimento, R.J., Sturm, N.R., 2006. Evolution and pathology in Chagas disease – a Review. Mem. Inst. Oswaldo Cruz 101, 463–491.

Wisnivesky-Colli, C., Schweigmann, N.J., Pietrokovsky, S.M., Bottazzi, V., Rabinovich, J.E., 1997. Spatial distribution of *Triatoma guasayana* (Hemiptera, Reduviidae) in hardwood forest biotopes in Santiago del Estero. Argent. J. Med. Entomol. 34, 102–109.