Phylogenetics Based on Partial ORF2 of Triatoma Virus in Triatomines Collected Over a Decade From Domiciliary Habitats

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ABSTRACT. The only virus sequenced and studied in triatomines is the Triatoma virus, from the Dicistroviridae family, which causes delayed development, reduced oviposition, and premature death of infected insects. With the goal of expanding the sequences already obtained in previous years and verifying if any changes occurred in their genomic sequences, 68 samples of triatomines from several provinces of Argentina were analyzed. Sixteen positive samples were obtained by Reverse Transcription (RT)-polymerase chain reaction using the VP3-VP1 subregion of open reading frame-2 as a diagnostic method; after sequencing, 11 samples were obtained from Triatoma infestans. These new sequences showed no significant differences in the analyzed regions, which were not grouped by species or habitat or geographical distribution. There were no differences when compared with the sequences found during 2002–2012, all obtained from the wild. We conclude that despite being an RNA virus, the different sequences show high homology.

Key Words: Triatoma infestans, domiciliary habitat, triatoma virus, Argentina

Materials and Methods

T. infestans Samples Collection. Samples of T. infestans were collected from different regions in 10 provinces of Argentina (Buenos Aires, Chaco, Córdoba, Formosa, La Rioja, Mendoza, San Luis, Santa Fe, Santiago del Estero, and Tucumán) between 2002 and 2012. Searches were conducted in peri-domicilries, cracks in adobe walls as well as in every deposit, corral, etc. Dislodging substances such as tetrathiem 0.2% were used to facilitate collection of the insects when necessary. The insects collected individually were transported to the laboratory in sterile plastic containers, each containing a folded piece of paper and capped with fine mesh screen. Subsequently, the insects were identified following Lent and Wygodzinsky (1979) and maintained at a temperature of 28 ± 1°C, with 60 ± 5% relative humidity and a photoperiod of 12:12 (L:D) h in special Centro de Estudios Parasitológicos y de Vectores (CEPAVE) facilities. There were a total of 68 samples that were stored at −80°C until use. Each sample contained 10 triatomines fecal material from each region.

Processing of Samples and RNA Extraction. Approximately 10 mg of fecal sample from the triatomines was homogenized with 200 μl of phosphate-buffered saline (PBS) in microtubes. RNA was extracted directly from fecal samples. A volume of 50 μl of samples resuspended in PBS was homogenized in 300 μl of TRIZOL reagent (Gibco-Invitrogen, M & M section), according to the manufacturer’s instructions. RNA concentration was determined by measuring absorbance at 260 nm (A260) in a spectrophotometer (Marti et al. 2008).

RT-Polymerase Chain Reaction (PCR) as Diagnostic Method. This first RT-PCR, called “diagnostic-PCR,” used primers TrVla (5’ TCA AAACTAACATCATCTCGG 3’) and TrVlb (5’ TTCAGCCT TATTCCTCCCC 3’), covering a region between VP3 and VP1, with an expected size of 832 bp. RT-PCR was performed following the methodology of Marti et al. (2008). The first TrV strain obtained from T. infestans captured in peri-domiciliary environments in 2001 (Guanaoco Muerto Dean Funes, Córdoba Province) was used as positive control (Susevich et al. 2012).
Results

Diagnostic PCR. In total, 68 samples were analyzed. The “diagnostic-PCR” using primers TrV1a and TrV1b gave the expected size of 832 bp. Sixteen samples were positive by this RT-PCR, and 11 of these samples could be sequenced—TIN3 to TIN13—(Table 1). To perform phylogenetic reconstruction of amino acids, 15 taxa were used with the NJ method and Bootstrap of 1,000 replicates with Poisson correction.

Sequences Included in This Work. Some previously published sequences were included to verify differences in host, environment, and geographical distribution. Names, province, year, and accession numbers are AF178440 (Czibener et al. 2000), TIN1-ARG (Côrdeiro 2002), TIN2-ARG (Chaco 2007), TDE1-ARG (Chaco 2007) and PCO1-ARG (Chaco 2005)—GenBank HM044313, HM044314, HM044312, and HM044315, respectively—(Susevich et al. 2012) and one sequence of T. delpontei isolated from a bird nest (Psittaciformes) from Chaco province in 2007 (TDE2-ARG accession number JQ657727).

Discussion

Our results indicate the widespread distribution of the virus, not only in wild species (Susevich et al. 2012) but also in peridomestic environments, thus increasing the likelihood of its potential use as a biological control agent against the vectors of Chagas disease. To date, the programs to control American trypanosomiasis are based exclusively on the use of chemicals in and around households, and no test has been conducted using TrV in the wild. The elucidation of their diversity and number, hosts, and pathogenic effects may reveal major roles of dicistroviruses in the biosphere (Bonning and Miller 2010). The prevalence of TrV in Argentina has been partially studied, however, in other areas, endemic for Chagas disease, such as other Latin America countries, is still unknown.

Structural and nonstructural viral proteins have been shown to be suitable targets for phylogenetic studies in other dicistroviruses. This study presents a phylogenetic analysis of the structural protein gene region of TrV sequences. We focused our investigations on the structural protein gene because this genomic region usually shows a higher degree of divergence than nonstructural genomic regions. Comparison of the complete genome sequences of central European acute bee paralysis virus (ABPV) genotypes and the reference strain did not result in significant divergences at any regions of the investigated strains (Bakonyi et al. 2002). Other investigations have shown that the Kashmir bee virus and ABPV of the honeybee can be broadly separated by different continents of origin but that it is more difficult to identify regional trends within each continent. Although nonstructural proteins

Table 2. Amino acid changes in the subregion of ORF2 from the sequences of Argentine TrV, with respect to reference strain AF178440 (3)

| Subregion Position | AA | Sequence |
|-------------------|----|----------|
| TrV 1a/1b         | R  | TIN9     |
| K                 | AF178440, TIN2, TIN4, TIN10, PCO1 |
| E                 | TIN1, TIN3, TIN5, TIN6, TIN7, TIN8, TIN11, TIN12, TIN13, TDE1, TDE2 |
| 161               | A  | AF178440, TIN1, TIN2, TIN3, TIN4, TIN5, TIN6, TIN7, TIN8, TIN9, TIN10, TIN11, TIN13, PCO1, TDE1, TDE2 |
| 191               | F  | TIN12    |
| L                 | AF178440, TIN1, TIN2, TIN3, TIN5, TIN6, TIN7, TIN8, TIN9, TIN10, TIN11, TIN12, TIN13, PCO1, TDE1, TDE2 |
| 250               | V  | TIN1     |
| L                 | AF178440, TIN1, TIN2, TIN3, TIN4, TIN5, TIN6, TIN7, TIN8, TIN9, TIN10, TIN11, TIN12, TIN13, PCO1, TDE1, TDE2 |
can be expected to have less variability within the genome, this is not always the rule (De Miranda et al. 2004). In the case of the black queen cell virus (BQCV), one study showed that the 5′-proximal third of ORF1 was the most variable region and contains several insertions and deletions (Reddy et al. 2013). However, other study showed that ORF1 or ORF2 should be useful to classify BQCV genotypes according to geographical origin (Noh et al. 2013). On the other hand, ORF2 showed better phylogenetic grouping corresponding to the geographical origin of the genotypes as in the case of ABPV (Tapaszti et al. 2009). These authors performed a phylogenetic analysis based on the structural proteins of ORF2 of BQCV (514 bp), which provided higher resolution than ORF1 and were able to separate strains from Poland, Austria, and Hungary. Moreover, other authors found two distinct lineages by analyzing the intergenic region and part of the ORF2 of Israeli Acute Paralysis Virus (Blanchard et al. 2008). At present, we are not able to compare sequences from different origins, because TrV has not yet been described in countries other than Argentina in nature. There is insufficient sequence data available from the database.

With respect to the sequence analysis described in this study, mutations were found at nucleotide level resulting in amino acid changes at various positions. In the region covered by the primers and TrV1a and TrV1b, changes consisted of 10 nt, corresponding to four amino acid changes. All the sequences obtained in this study were closely related, even though they had been isolated from different triatomines, different environments and provinces, and in different years.

We have been faced with the difficulty of the low rate of recovery of RNA from the stool samples of the triatomines analyzed. We attribute this primarily to the fact that, despite the extreme care taken to manipulate the materials to minimize the risk of contamination, which causes RNA to be inactivated, the latter could not be recovered in its entirety from the samples preserved in nuclease free water at –80°C. Stool samples, although extremely useful, are not available for subsequent uses. Moreover, these observations raise the question of the specificity of the primers used or whether the RNA obtained from different samples was not obtained as single strand.

Because there is no clear geographical, temporal, or ecological separation between the sequences to support their clear phylogenetic separation based on ORF2 in our limited number of sequences obtained, future research into RT-PCR-based diagnostics should target regions of the ORF1 or the intergenic region, because these may have a high level of variation and allow us to classify genotypes according to geographical origin. A more comprehensive genome analysis should be performed in the future to identify and classify TrV isolates from different geographic regions. Moreover, further studies are required to increase our knowledge and understanding about the presence of TrV in Latin American countries.

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Fig. 1. Phylogenetic trees obtained by the NJ method from the analysis of nucleotides (A) and putative amino acids (B) from the TrV1a and TrV1b subregion of Argentine and reference sequences. Numbers above branches indicate support values.

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