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BRIEF REPORT

First description of hemagglutination by a virus belonging to the family *Dicistroviridae*

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Abstract Triatoma virus is the only virus whose genome has been sequenced and studied in triatomines. It belongs to the family *Dicistroviridae*. In order to detect whether TrV has the ability to agglutinate erythrocytes of domestic and laboratory animals, we performed a hemagglutination assay. Positive hemagglutination was found for red blood cells of guinea pigs. The HA assay could be used as a titration method, at least for purified viral particles obtained from triatomine stool. This is the first record of hemagglutinating properties for *Dicistroviridae*.

Keywords Triatoma virus · Hemagglutination · Domestic and laboratory animals · Triatominae

Triatoma virus (TrV) is a single-stranded RNA virus, initially classified as a member of the family *Picornaviridae* [13], along with 20 other viruses described in insects [11, 12]. Studies based on the analysis of the RNA nucleotide sequence of TrV suggested that this virus had characteristics that distinguished it from members of other known virus families. For that reason, in 2002 this virus was assigned to the newly established family *Dicistroviridae*, with a single genus (*Cripavirus*) including, in addition to *Triatoma virus*, eight other species: *Drosophila C virus* (DCV), *Aphid lethal paralysis virus* (ALPV), *Cricket paralysis virus* (CrPV), *Black queen cell virus* (BQCV), *Himetobi P virus* (HiPV), *Plautia stali intestine virus* (PSIV), *Rhopalosiphum padi virus* (RhPV), and *Homolodisca coagulata virus-1* (HoCV-1) [7]. In 2010, Bonning and Miller proposed a second genus, *Aparavirus*, which now includes six species: *Acute bee paralysis virus* (ABPV), *Taura syndrome virus* (TSV), *Kashmir bee virus* (KBV), *Solenopsis invicta virus-1* (SINV-1), *Israeli acute paralysis virus* (IAPV), and *Mud crab virus* [3]. There are at least fourteen families with representatives that are viral pathogens of invertebrates, and some of these families contain viruses that have been investigated as potential biological control agents for insects. Members of the family *Dicistroviridae* has been proposed for this purpose [5]. As described by Bonning and Miller [3], eight members of this family are recognized as pathogenic to insect pests of crops (CrPV, ALPV, RhPV, PSIV, HiPV, HoCV-1, SINV-1) or of medical importance (TrV and SINV-1). Therefore, these viruses could be used as biological control agents. TrV is important because it is one of the best studied pathogens of triatomines that are vectors of Chagas disease. In the absence of a vaccine or effective therapeutic drugs to combat this disease, in Argentina the fight against it is almost exclusively limited to the management of the populations of its main vector, *Triatoma infestans* (Klug), in attempts to reduce their density. Notably, although Chagas disease is mainly distributed in Latin America countries, recent decades have witnessed more frequent occurrences in the United States, Canada, many European countries and some Western Pacific countries [17].

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TrV particles are naked, about 30 nm in diameter, and the capsid is composed of three major proteins VP3 (31.9 kDa), VP1 (29.7 kDa) and VP2 (28.5 kDa) and a precursor protein VP0 (38 kDa) [1]. According to Czibener et al. [4], TrV RNA has two different open reading frames (ORF1 and ORF2) flanked by non-coding regions. These ORFs code for non-structural and structural proteins of the virus, respectively. The ORFs are separated by an intergenic noncoding region of 172 nucleotides, and at both the 5′ and 3′ end there are untranslated regions (UTRs) of 550 and 295 nucleotides, respectively. The IG-IRES activity is highly dependent on the host cell; not only the integrity of the IRES allows viral replication, but cellular factors and/or viral infections are needed to achieve effective viral replication and mature virion packaging [10].

In order to isolate and characterize this virus and perform an in-depth exploration of its biology, with the view of obtaining viral mass, its replication was attempted in different cell lines such as Sf9, Sf21, High Five and C6/36. Inoculation of TrV into insect cell cultures, either as whole purified particles or as purified RNA, in some cases resulted in probable signs of infection or cytopathic effect (CPE), which was visible in the first 24-48 h. This effect disappeared after successive passages. These experiments were carried out using diverse methods: RNA electroporation, addition of Lipofectin, direct inoculation of purified virus, and electroporation of viral particles. Due to the negative results obtained with these methods, we experimented with the hemagglutination technique (HA). The literature shows no references for this; however, the HA technique could be used at least as a titration method for purified viral particles from triatomine stool.

Hemagglutination is one of the most common methods for indirect characterization and quantification of viral particles or viral antigens, either by hemagglutinating suspension or using the microhemagglutination technique. Some viruses, such as influenza virus [8], enterovirus [2], and torovirus, have been reported to hemagglutinate red blood cells from different species [15]. The hemagglutination inhibition technique has been used to detect antibodies against yellow fever virus, varicella zoster virus, influenza virus, parainfluenza virus [6], and dengue virus [16]. So far, little is known about the properties of members of the family *Dicistroviridae*. Furthermore, hemagglutinating properties have been found in some enterovirus of the family *Picornaviridae,* where TrV was initially assigned. The goal of this study was to detect if TrV possesses the ability to agglutinate erythrocytes from domestic and laboratory animals.

Viral particles were purified from insects obtained from an experimentally TrV-infected colony of *T. infestans*, maintained at the Centro de Estudios Parasitológicos y de Vectores (CEPAVE). A total of 30 infected adult insects were dissected, and their midguts were homogenized, clarified and resuspended in NMT buffer (0.01 M NaCl, 0.001 M $MgCl_2$ and 0.04 M Tris-HCl, pH 7.4) and layered on top of a continuous sucrose gradient (10–30 %, w/v) [9, 13].

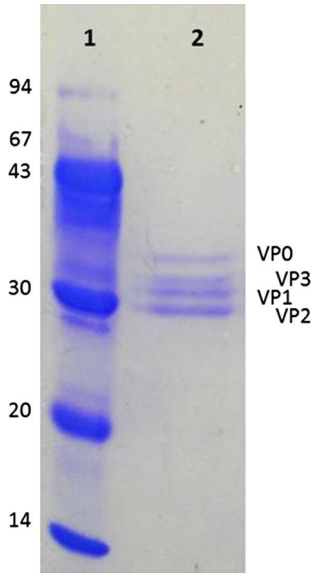

Fig. 1 SDS-PAGE of TrV that was purified by sucrose gradient centrifugation, stained with Coomassie blue. Lane 1, molecular weight marker; lane 2, TrV structural proteins

After centrifugation at 100,000*g* for 3 h in a Beckman SW28.1 rotor, the gradient was fractionated in 0.5-mL aliquots. The optical density of each aliquot was then measured at both 260 nm and 280 nm using a Bio Rad Smart SpecTM 3000 spectrophotometer. Then, the selected aliquots were loaded onto 12.5 % polyacrylamide SDS-PAGE gels and stained with Coomassie blue to estimate the purity and concentration of the samples. 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. Finally, in order to confirm their identity, TrV proteins were separated in a 12.5 % SDS-PAGE gel and stained as described above [9]. The concentration of the selected sample obtained after purification was 0.2 mg/ml of virus (Fig. 1).

Red blood cells (RBCs) used for different HA assays were obtained from cow, horse, sheep, goat, dog, rabbit, rat, mouse, peacock, goose, pig, cat, and chicken (domestic and laboratory animals). Furthermore, adult human type 0 and A blood was used. RBCs were removed by venipuncture and collected in Alsever's solution in a 10–15 % volume. After gently mixing by inversion, the suspensions were stored at 4 °C until use. RBCs were washed in phosphate-buffered saline (PBS), pH 7.3, and centrifuged at 250×*g* for 10 min to pellet the cells. The supernatant

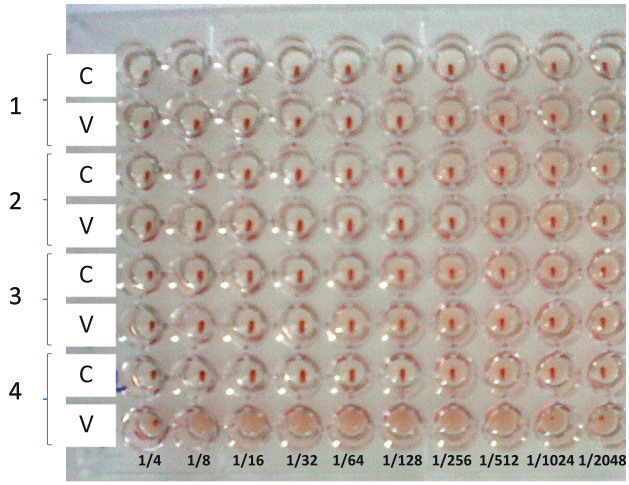

Fig. 2 Microhemmaglutination of TrV using different red blood cells. C, control (Red blood cells + PBS); V, TrV; 1 to 4, red blood cells from cow, horse, goose and guinea pig respectively. Viral dilutions are indicated at the bottom of the plate

was removed and cells were resuspended in PBS and centrifuged again at 250×*g* for 10 min. This step was repeated at least three times, or until the supernatant was clear.

Tests were carried out in 96-well V-bottomed plates. In the first column, each well was filled with 25 µl of purified virus and 75 µl of PBS as diluent (1/4 dilution). In columns 2 to 12, each well contained 50 µl of PBS, and the test included a continuous line for negative control. Then, twofold dilution series were done from column 1 to 12 (50 µl). Finally, 50 µl of 0.5 % RBCs was added to each well, and the plates were incubated for 1 h at room temperature. A result was considered positive when agglutination was equal or greater than the size of 50 % of the well bottom. The highest dilution (1/1024) of virus showing HA activity was designated as the HA titer (1024) or one HA unit.

In this study, TrV showed complete agglutination of guinea pig RBCs (Fig. 2). No other RBCs were agglutinated by TrV; however, autoagglutination was observed in some RBCs, namely human O type, goat, rabbit, pig and rat. As mentioned above, TrV was originally assigned to the family *Picornaviridae*. Tests on the foot-and-mouth disease (FMD) virus, which belongs to this family, have shown that it does not cause agglutination by itself. Reverse genetics has been used to introduce hemagglutinin into the capsid of FMD virus [14]. In our case, we were able to detect hemagglutination by TrV and to titrate until reaching a 1/1024 dilution.

This is the first report of hemagglutination by a member of the family *Dicistroviridae*. We consider this important, given that it has not been possible to replicate TrV in cell lines, and the only known method for its titration requires spectrophotometry. Furthermore, this is the first report of hemagglutination properties for TrV.

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