Double-Stranded RNA Viral Infection in Cuban *Trichomonas vaginalis* Isolates

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***Trichomonas vaginalis* can be infected with double-stranded RNA (dsRNA) viruses designated *T. vaginalis* virus (TVV), which may have important implications for trichomonal virulence and disease pathogenesis. We tested for TVV in 40 fresh *T. vaginalis* isolates from Cuban patients by total extraction of nucleic acids (DNA and RNA). TVV was detected in 22 (55%) of the 40 *T. vaginalis* isolates. This gives an estimate of the infection rate of Cuban *T. vaginalis* isolates by the dsRNA virus. Future research should focus on the association between trichomonosis symptoms and the presence of TVV.**

Key Words: *Trichomonas vaginalis*, *Trichomonas vaginalis* virus, dsRNA virus.

Trichomonas vaginalis is the most prevalent nonviral sexually transmitted disease in the world [1]. *Trichomonas vaginalis* infection has been associated with premature rupture of membranes and premature delivery in pregnant women [2]. Trichomonosis has also been associated with an increased risk of human inmunodeficiency virus (HIV) acquisition and transmission [3]. Many *T. vaginalis* isolates are infected by a small double-stranded RNA (dsRNA) virus designated *T. vaginalis* virus (TVV). This infection causes up-regulation of synthesis and surface expression of a highly immunogenic protein, P270 [4] and significant differences in the total protein composition of the parasite [5,6]. The presence of TVV is also associated with differential qualitative and quantitative expression of cysteine proteinases [7]. Thus, TVV induces various phenotypic changes that may impact on *T. vaginalis* virulence [5,7]. The presence or absence of a dsRNA virus defines two naturally-occurring types of isolates of *T. vaginalis* [8]. Isolates composed of virus-negative trichomonads are termed "type I", whereas those with virus-positive parasites are termed "type II". We evaluated the prevalence of type II isolates in adolescents attended at two gynecobstetrics hospitals in Havana City, Cuba.

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Material and Methods

Forty fresh *T. vaginalis* isolates, collected from infant-juvenile-age adolescents, attended at the sexually transmitted infections clinic in the gynecobstetrics hospitals "Ramón González Coro" and "Eusebio Hernández" of Havana City, were analyzed. Various laboratory tests were carried out, to determine if other sexually-transmitted microorganisms were present. *Trichomonas vaginalis* isolates were obtained from vaginal exudates, and they were cultivated at 37°C in TYI-S-33 Diamond's medium [9], supplemented with 10% heat-inactivated calf serum. Successive transfers in TYI-S-33 medium supplemented with antibiotics (50 μg/mL gentamicine, 100 U/mL penicillin, 30 μg/mL streptomycin sulfate) and an antifungal compound (60 μg/mL nistatine) were carried out to produce the axenic isolates. Aliquots of $2x10^6$ cells of *T. vaginalis* in the exponential phase of *in vitro* growth were placed in a –70ºC freezer for later evaluation of dsRNA viral infection.

The *T. vaginalis* pellet from each isolate was washed with 1x PBS. Later, they were centrifuged at 8000 x g for 10 min at 4°C. The pellet was resuspended in 300 µL of lysis buffer (50 mM tris-HCl pH 8.25, 25 mM EDTA, 25 mM NaCl, 1% SDS). The suspension was incubated with 100 µg/mL of proteinase K (Boehringer Mannheim, Germany) for 2 h at 56°C. Nucleic acids were extracted with an equal volume of a mixture of phenol-chloroform-isoamyl alcohol (25: 24:1) and with chloroform-isoamyl alcohol (24:1), with centrifugations at 8,000 x g for 10 min at 4°C. The nucleic acids were precipitated by adding two volumes of absolute ethanol containing 3 M sodium acetate, kept at –20°C for 30 minutes, and then pelleted by centrifugation at 10,000 x g for 20 min. The pellet was washed in 70% ethanol. After air-drying, the DNA-RNA was dissolved in 25 µL tris-EDTA buffer (TE) (1 mM tris-HCl pH 8.0, 1mM EDTA pH 8.0). The nucleic acids were stained with ethidium bromide (0.5 mg/mL) after electrophoresis in 1% agarose gel in TBE buffer (0.5x TBE, 0.045 M tris-borate, 0.001 M EDTA) and visualized using a UV transilluminator (Macrovue 2011, LKB).

Results

The *T. vaginalis* isolates were screened for the presence of TVV by electrophoresis of simultaneously-extracted DNA and RNA. Among the 40 isolates examined, 22 were type II and exhibited a fragment of approximately 4.6 kbp, which was regarded to be dsRNA TVV virus (Figure 1).

Nuclease digestions confirmed the double stranded RNA nature of these fragments. DNase I did not degrade the fragments, whereas RNase A did.

Discussion

The TVV was initially described as having a non-segmented 5.5 kbp dsRNA genome [8]; further investigation suggested the possibility of several dsRNA segments in virus-harboring *T. vaginalis* isolates.

Khoshnan and Alderete [10] described at least three unique dsRNA segments, with sizes ranging from 4.8 to 4.3 kbp. The results of their analyses suggest a segmented nature of the *T. vaginalis* dsRNA virus, or possibly infection of this protozoan by several different viruses. Co-infections by 0.5 kbp small dsRNAs, together with TVV genome 4.6 kbp dsRNA, is commonly obtained in *T. vaginalis* isolates [11].

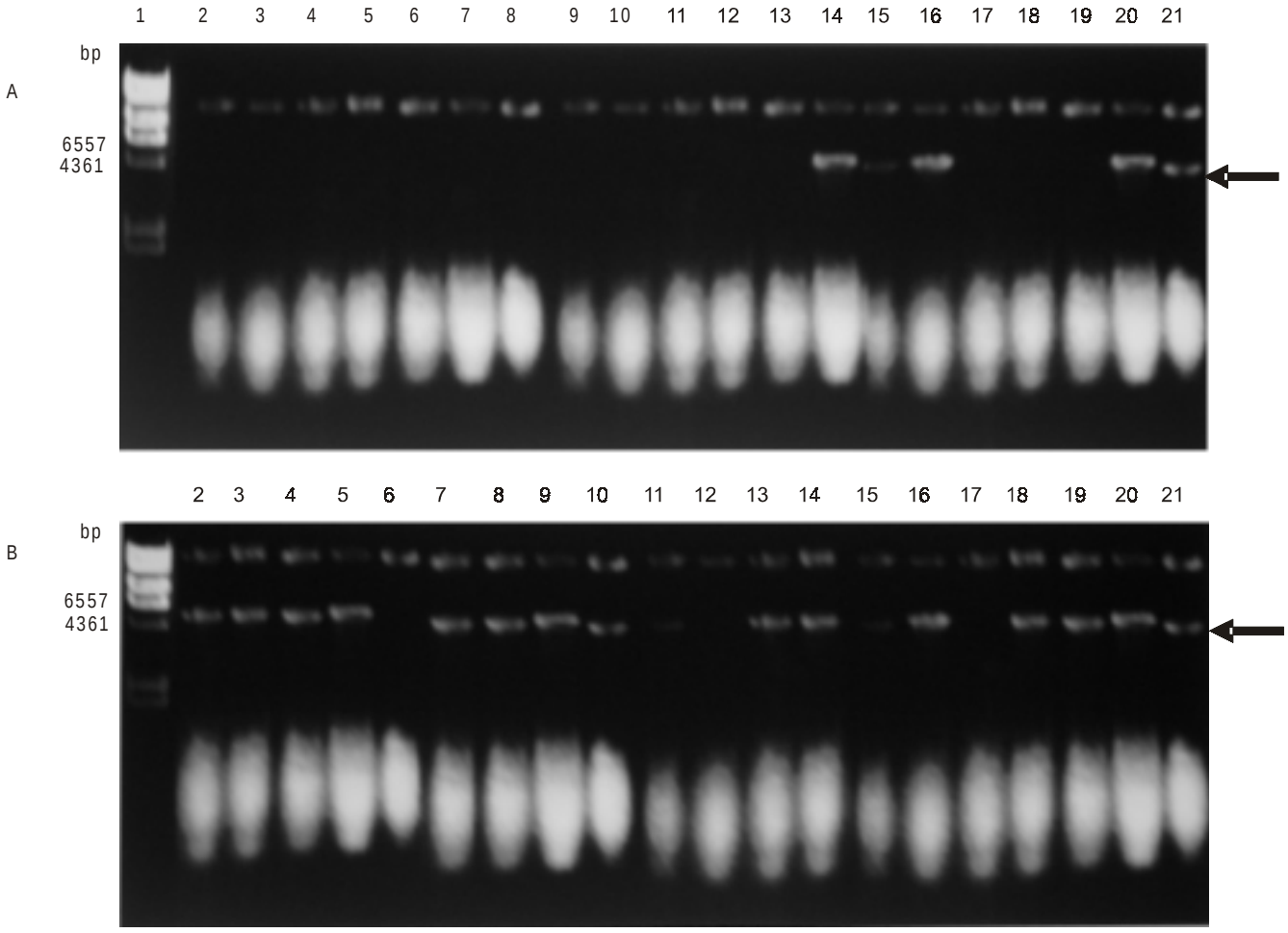
Benchimol et al. [12], described a *T. vaginalis* isolate with a virus population composed of different viral-like particles with varying sizes (33 - 200 nm) and shape (filamentous, cylindrical, and spherical particles), which is evidence for infection with one or several distinct dsRNA viruses. The TVV genome encodes for capsid protein and RNA-dependent RNA polymerase [13, 14]. Alderete et al. [15] found the genetic variations that exist within the capsid protein gene; this may explain the diversity in this non-segmented dsRNA virus.

The dsRNA derived from CsCl-purified virus gives bands that migrate identically to the dsRNA in the total nucleic acid preparation; no virus has been detected within the virus-minus organism [12]. We found only a fragment of around 4.6 kbp in all of the type II *T. vaginalis* isolates, when we analyzed the dsRNA virus in a total nucleic acid preparation separated in a 1% agarose gel and stained by ethidium bromide.

TVV is found in approximately 50% of clinical isolates [5]. The TVV infection rate in our study (55%) was similar to previous data and other reports. Vanacoca et al. [16] and Hampl et al. [17] found an infection rate of 44 % when they analyzed 20 isolates from different geographic origins. Snipes et al. [18] found a 50% infection rate when they analyzed 109 isolates in the USA. Others studies reported a higher rate, 81.9 and 75%, in analyses of 72 South African *T. vaginalis* isolates [19] and 28 USA (Baltimore) isolates [20], respectively.

This is the first report of TVV in Cuban *T. vaginalis* isolates, and it indicates an estimated infection rate in Cuba by the dsRNA virus. Future research should focus on the association between trichomonosis symptoms and the presence or absence of TVV.

Figure 1. Agarose gel electrophoresis (1 %) of nucleic acids from fresh *Trichomonas vaginalis* clinical isolates. Presence or absence of dsRNA virus (TVV). (A) Lane 1: Molecular weight marker 1 kb (Promega, USA), Lane 2- 21 Clinical isolates: C91, C129, C175, C237, C239, C350, C353, C356, C358, C361,A59, A163, A170, A185, C9, C12, C15, C98, C334, C344. (B) Lane 1: Molecular weight marker 1 kb (Promega, USA), Lane 2-21 Clinical isolates: A5, A42, C147, C76, A69, C190, C240, C247, C187, C206, A47, A66, C308, C313, C173, C348, C349, C355, C351, C352. The arrow indicates the position of the double-stranded RNA virus.



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