Double stranded RNA virus in South African *Trichomonas vaginalis* isolates

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**Aims:** To screen *Trichomonas vaginalis* isolates from South Africa for the presence of a small double stranded RNA virus designated *T vaginalis* virus (TVV).

**Methods:** TVV was detected by simultaneous extraction of DNA and RNA, and its presence confirmed by electron microscopy and nuclease digestions.

**Results:** TVV was detected in 59 of 72 (81.9%) isolates.

**Conclusions:** These results indicate a possible higher infection rate of South African *T vaginalis* isolates by the double stranded RNA virus than has been reported for isolates elsewhere.

The organism *Trichomonas vaginalis* is a protozoan parasite that is responsible for one of the most prevalent sexually transmitted diseases (STDs). *Trichomonas vaginalis* infections have been associated with an enhanced predisposition to other STDs, including human immunodeficiency virus, premature labour during pregnancy, and a higher risk of cervical neoplasia. Studies in South Africa on presumably asymptomatic groups of women reported very high trichomoniasis rates of 20% to 49%. It is not clear whether this is a consequence of socioeconomic conditions and/or differences in the virulence of the parasite strains. Interestingly, many *T vaginalis* isolates are infected by a small double stranded RNA virus designated *T vaginalis* virus (TVV). Changes in the expression of a prominent immunogen (P270) and significant differences in the total protein composition of the parasite have been associated with infection by the virus, but currently there is no evidence that TVV alters the pathogenicity of *T vaginalis*.

Many *Trichomonas vaginalis* isolates are infected by a small double stranded RNA virus designated *T vaginalis* virus

The aim of our study was to determine whether South African *T vaginalis* strains harbour the double stranded RNA virus and also to determine the rate of infection.

**MATERIALS AND METHODS**

Fresh clinical isolates of *T vaginalis* collected at hospitals in Ga-Rankuwa and Cape Town, South Africa, were inoculated into Diamond’s medium and maintained as axenic cultures. Simultaneous extraction of DNA and RNA was performed according to the method described by Chou and Tai. Nuclease digestions were performed on 1.5 µg of extracted nucleic acids in four separate reactions with: (1) RNase A (100 µg/ml); (2) and (3) RNase T1 (10 U/µg) in 500mM and 50mM NaCl, respectively; and (4) RNase free DNase I (10 U/µg) (all nucleases were from Roche Diagnostics, Mannheim, Germany). Reactions were incubated for 30 minutes at 37°C and analysed on a 1% agarose gel.

For electron microscopy, 15 ml aliquots of the *T vaginalis* cultures were centrifuged for 10 minutes at 1000 × g. The supernatant was then centrifuged at 35 000 rpm (154 693 × g (average), 217 874 × g (maximum) for two hours (Beckman L7–65, SW40 rotor; Beckman Coulter Inc, Fullerton, California, USA). Pellets were dissolved in 100 µl water and mixed with 100 µl of 2% phosphotungstic acid. Stained specimens were loaded on to a formvar/carbon coated copper grid and examined with a Philips 301-TEM (Philips, Eindhoven, the Netherlands).

**RESULTS AND DISCUSSION**

South African strains of *T vaginalis* were screened for the presence of TVV by electrophoresis of simultaneously extracted DNA and RNA. Of the 72 strains examined, 59 exhibited a fragment of approximately 4.5 kilobase pairs, which was regarded as TVV (fig 1).

**Abbreviations:** TVV, Trichomonas vaginalis virus; STDs, sexually transmitted diseases
Nuclease digestions confirmed the double stranded RNA nature of these fragments. RNase T1 at high salt concentrations and DNase I did not degrade the fragments, whereas RNase A and RNase T1 at low salt concentrations did.

In addition, two strains of *Trichomonas vaginalis* possessing the fragment of interest and two not possessing this fragment were further investigated with transmission electron microscopy. Characteristic virus-like particles of 32 nm were seen only in the strains containing this fragment (fig 2).

The TVV infection rate in our study (81.9%) is much higher than rates reported by others. Infection rates of 50.4% and 44.4% were found in *Trichomonas vaginalis* isolates mainly from the USA and the Czech Republic, respectively. Although it is not clear at present whether TVV infection contributes to hypovirulence or hypervirulence, the carriage of TVV could be a reason for the high incidence of *T vaginalis* infections in South Africa. However, more studies to determine the effect of TVV on the pathogenesis and virulence of *T vaginalis* are clearly indicated.

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**Take home messages**

- *Trichomonas vaginalis virus* (TVV) was found in 59 of 72 (81.9%) *Trichomonas vaginalis* isolates from South Africa.
- This rate of infection with TVV is higher than the rates that have been reported for isolates elsewhere and it is possible that the carriage of TVV is related to the high incidence of *T vaginalis* infections in South Africa.
- More studies to determine the effect of TVV on the pathogenesis and virulence of *T vaginalis* are needed.

**REFERENCES**


**Figure 2** Electron micrograph of negatively stained *Trichomonas vaginalis* strain 204. The arrows indicate the virus-like particles.