

SHORT REPORT

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

B Weber, T M Mapeka, M A Maahlo, A A Hoosen

J Clin Pathol 2003;56:542-543

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,¹ premature labour during pregnancy,² and a higher risk of cervical neoplasia.³ Studies in South Africa on presumably asymptomatic groups of women reported very high trichomoniasis rates of 20% to 49%.⁴ 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).⁵ Changes in the expression of a prominent immunogen (P270) and significant differences in the total protein composition of the parasite^{6,7} 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.⁸ 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.

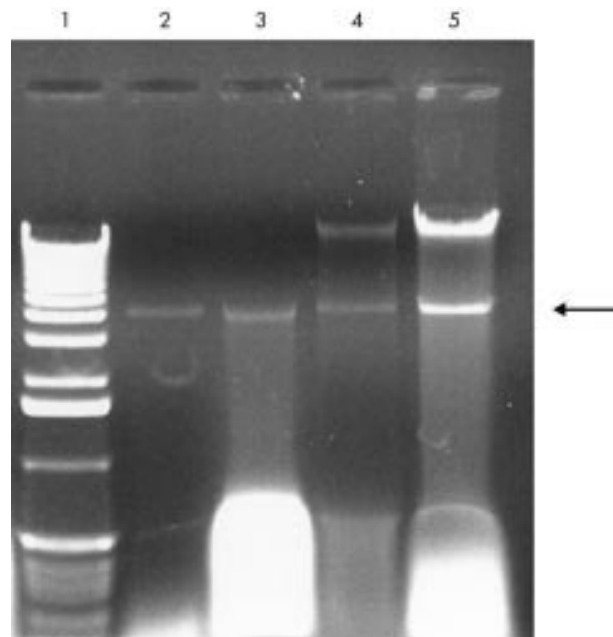


Figure 1 Agarose gel electrophoresis of *Trichomonas vaginalis* isolates that harbour *T vaginalis* virus. Lane 1, 1 kb ladder; lane 2, Triton soluble fraction of strain 204; lane 3, Triton soluble fraction of strain 105; lane 4, Triton insoluble fraction of strain 204; and lane 5, Triton insoluble fraction of strain 105. The arrow indicates the position of the double stranded RNA bands.

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

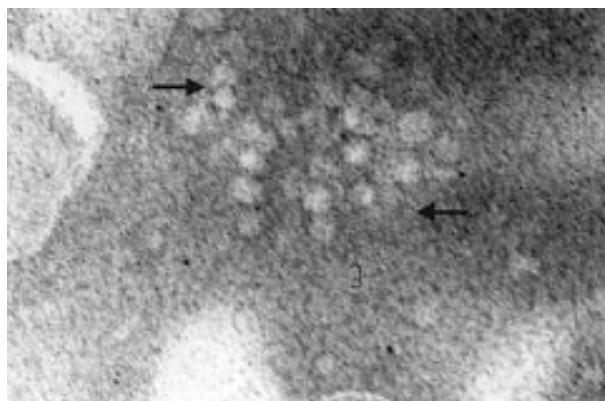


Figure 2 Electron micrograph of negatively stained *Trichomonas vaginalis* strain 204. The arrows indicate the virus-like particles.

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 *T 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 *T vaginalis* isolates mainly from the USA⁹ and the Czech Republic,¹⁰ 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.

ACKNOWLEDGEMENTS

This study was supported by an IRDP grant (GUN: 2038667) of the National Research Foundation of South Africa. We are grateful to Mr H Basetse for his help in electron microscopy.

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

Authors' affiliations

B Weber, T M Mapeka, M A Maahlo, A A Hoosen, Department of Medical Microbiology, Medical University of Southern Africa, PO Box 211, Medunsa 0204, South Africa

Correspondence to: Dr B Weber, Department of Medical Microbiology, Medical University of Southern Africa, PO Box 211, Medunsa 0204, South Africa; weber@anaesthesie.uni-kiel.de

Accepted for publication 6 February 2003

REFERENCES

- 1 **Sorvillo F**, Kerndt P. *Trichomonas vaginalis* and amplification of HIV-1 transmission. *Lancet* 1998;**351**:213–14.
- 2 **Cotch MF**, Pastorek JG, Nugent RP, *et al*. *Trichomonas vaginalis* associated with low birth weight and preterm delivery. The vaginal infections and prematurity study group. *Sex Transm Dis* 1997;**24**:353–60.
- 3 **Zhang ZF**, Begg CB. Is *Trichomonas vaginalis* a cause of cervical neoplasia? Results from a combined analysis of 24 studies. *Int J Epidemiol* 1994;**23**:682–90.
- 4 **Hoosen AA**, Quinlan DJ, Moodley J, *et al*. Sexually transmitted pathogens in acute pelvic inflammatory disease. *S Afr Med J* 1989;**76**:251–4.
- 5 **Wang AL**, Wang CC. The double-stranded RNA in *Trichomonas vaginalis* may originate from virus-like particles. *Proc Natl Acad Sci U S A* 1986;**83**:7956–60.
- 6 **Wang A**, Wang CC, Alderete JF. *Trichomonas vaginalis* phenotypic variation occurs only among trichomonads infected with the double-stranded RNA virus. *J Exp Med* 1987;**166**:142–50.
- 7 **Liu HW**, Chu YD, Tai JH. Characterization of *Trichomonas vaginalis* virus proteins in the pathogenic protozoan *T. vaginalis*. *Arch Virol* 1998;**143**:963–70.
- 8 **Chou CF**, Tai JH. Simultaneous extraction of DNA and RNA from nuclease-rich pathogenic protozoan *Trichomonas vaginalis*. *Biotechniques* 1996;**20**:790–1.
- 9 **Snipes LJ**, Gamard PM, Narcisi EM, *et al*. Molecular epidemiology of metronidazole resistance in a population of *Trichomonas vaginalis* clinical isolates. *J Clin Microbiol* 2000;**38**:3004–9.
- 10 **Vanacova S**, Tachezy J, Kulda J, *et al*. Characterization of trichomonad species and strains by PCR fingerprinting. *J Eukaryot Microbiol* 1997;**44**:545–52.



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

B Weber, T M Mapeka, M A Maahlo, et al.

J Clin Pathol 2003 56: 542-543

doi: 10.1136/jcp.56.7.542

Updated information and services can be found at:

<http://jcp.bmj.com/content/56/7/542.full.html>

References

These include:

This article cites 10 articles, 4 of which can be accessed free at:

<http://jcp.bmj.com/content/56/7/542.full.html#ref-list-1>

Article cited in:

<http://jcp.bmj.com/content/56/7/542.full.html#related-urls>

Email alerting service

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:

<http://group.bmj.com/group/rights-licensing/permissions>

To order reprints go to:

<http://journals.bmj.com/cgi/reprintform>

To subscribe to BMJ go to:

<http://group.bmj.com/subscribe/>