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REVIEW

Prevention or treatment: The benefits of *Trichomonas vaginalis* vaccine

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Summary Trichomoniasis (infection with *Trichomonas vaginalis*) is the most common non-viral sexually transmitted disease (STI) in the world. Although treatment is available, most cases occur in developing countries, where accessing healthcare is difficult and facilities are limited. Additionally, infection is often asymptomatic and as such goes untreated, creating reservoirs of *T. vaginalis* that allow the disease to spread within the community. Because of this there has been little success in controlling the incidence of trichomoniasis, especially amongst the underprivileged. The development of a vaccine against *T. vaginalis* could reduce the human costs (pregnancy complications, infertility), medical costs (repeated doctor visits, increased susceptibility to human immunodeficiency virus (HIV) infection), and societal costs (stigma of STI, cycles of untreated infection) associated with trichomoniasis.

Contents

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Trichomonas vaginalis and trichomoniasis

Sexually transmitted infections (STI) and their sequelae are among the top five reasons that people in developing countries seek medical treatment. Amongst women of child-bearing age (15—49), STI (excluding human immunodeficiency virus (HIV) infection) are second only to complications in pregnancy and childbirth as a cause of morbidity and mortality [1]. The World Health Organization (WHO) estimates that there are about 340 million cases of curable STI annually, although high rates of asymptomatic infection and reluctance to seek treatment for genital disease may lead to under-reporting. Of cases of the four major curable STI, approximately 3.5% (12 million) are of syphilis, 18.2% (62 million) are of gonorrhea, 27.1% (92 million) are of chlamydia, and 51.2% (174 million) are of trichomoniasis [2]. This makes infection with *T. vaginalis*, a parasitic protozoan that is the causative agent of trichomoniasis, the most common non-viral STI in the world, with the vast majority of infections occurring in developing countries.

T. vaginalis is the only member of the family Trichomonadidae known to cause disease in humans. Infection is spread by sexual contact and is confined to the genital tract [3]. Trichomoniasis in women is a disease of the reproductive years, although in contrast to other non-viral STI where infection is most prevalent in younger women (ages 15—25), the highest rates of *T. vaginalis* infection are usually found in older women (ages 35—40) [4,5]. This is hypothesized to be because untreated trichomoniasis is a chronic condition. Several studies examining *T. vaginalis* infection in women have been unable to show significant resolution of infection without antibiotic treatment, and as such the duration of infection is generally classified as indefinite [6]. About 50% of women with *T. vaginalis* infection will be asymptomatic, but about one-third of these will develop symptoms within 6 months [3]. Symptomatic cases of trichomoniasis generally present with pruritis, dyspareunia, and vaginal discharge, as well as vaginitis and vulvitis in more severe cases [3]. Some of the most serious complications occur if an infection is present during pregnancy. Trichomoniasis can lead to premature rupture of the placental membranes and result in premature labour and low birth-weight babies [3,7]. With pregnancy and childbirth being the leading cause of death for women globally, an infection that can further endanger the welfare of mother and child cannot be ignored.

Once considered to be mainly a disease of women, a number of more recent reports have stated that trichomoniasis rates among men, especially those who are partners of infected women, are much higher than previously suspected [8,9]. *T. vaginalis* infection in men is usually asymptomatic. Symptomatic infection presents as urethritis (dysuria and discharge) similar to other non-gonococcal infections [3]. Unlike in women, trichomoniasis in men will usually resolve without treatment, although there is some debate as to the duration of infection without intervention (2 weeks to 4 months) [10].

T. vaginalis infection does not lead to long term immunity and re-infection can readily occur. The high rate of asymptomatic infection, particularly in men, means that many people unknowingly harbour the parasite and act as carriers, spreading the infection in their community.

Problems with current treatment and prevention strategies

T. vaginalis infection is treated with metronidazole. Metronidazole belongs to the 5-nitroimidazole drug family and it and related compounds, such as tinidazole and seconidazole, are reported to have about a 95% success rate in curing trichomoniasis [11]. The preferred treatment regimen is a one-time oral 2 g dose, which combines maximum patient compliance with a minimum amount of the drug (Centre for Disease Control and Prevention (CDC) guidelines). Metronidazole is inexpensive and generally well tolerated. Severe adverse reactions to standard doses are rare and usually a result of allergic reaction to nitroimidazoles [12].

Although metronidazole is relatively cheap and effective, there are still significant barriers to reducing the global burden of *T. vaginalis* infections. Drug resistance to metronidazole has been reported in 2.5—9.6% of trichomoniasis cases [13,14]. In the absence of non-nitroimidazole alternatives to treatment, cure can only be achieved by increasing dosages of metronidazole [12]. Higher doses lead to increased and sometimes intolerable side effects, which may necessitate cessation of treatment and chronic infection.

There is also a huge disparity in the diagnosis and treatment of STI in developed vs developing countries. In industrialized countries with relatively easy access to health care, people who experience abdominal or genitourinary discomfort usually seek medical attention quickly. Testing is available to establish the identity of the pathogen in question, and the appropriate antibiotic is prescribed.

In developing countries, access to health care may be limited and significant travel may be required to reach the nearest medical facility. As such, many patients are unlikely to seek out medical care for a ''nuisance'' infection (as trichomoniasis has been described). Additionally, it is often not technologically or economically feasible to test for specific pathogens, so patients presenting with genitourinary symptoms are given antibiotics appropriate to the most likely cause of infection. This syndromic treatment is very cost effective if the pathogen is correctly diagnosed, but it is not necessarily successful in reducing the incidence of *T. vaginalis* infection because the symptoms of trichomoniasis (discomfort and discharge in women, urethritis in men) are often similar to other bacterial or fungal infections and the antibiotics commonly used to treat these infections are ineffective against *T. vaginalis*. One study model has shown that even in poorer areas it would be cost effective to add metronidazole as part of syndromic management to patients presenting with probable STI, when compared with the cost of repeated doctor visits for failed cure [15]. However, syndromic treatment still fails to address the issue of asymptomatic infections which act as a reservoir for the parasite.

Although the partners of patients with trichomoniasis are often infected, high rates of asymptomatic infection mean that they do not always seek treatment and as such are likely to re-infect a partner who has been treated. Partner treatment is recommended to prevent re-infection, but is generally not mandated as standard of care [16]. As such, partner treatment relies on the willingness of a patient with trichomoniasis to reveal their condition to their partner(s) and the reciprocal willingness of the partner to seek out testing/treatment. Even in studies where the patient is provided with a suitable dose of metronidazole to provide prophylaxis for their partner, it was shown that unwillingness of the patient to reveal their state of infection or refusal of the partner to accept treatment meant that consistent partner treatment may not be achieved [16].

From a public health standpoint, the issue of partner treatment and re-infection is particularly difficult when dealing with sex trade workers and their clients. Studies have shown 13—45% of female sex workers (FSW) are infected with *T. vaginalis* [17]. Contrary to the general population, younger FSW are more likely to have trichomoniasis than older FSW, probably because they do not or cannot enforce consistent condom use by clients [18]. This means that not only is the risk of contracting and spreading *T. vaginalis* greater, but increases the likelihood of inflammation and pregnancy related complications. With the male to female rate of transmission being estimated at 85—100%, FSW who do not use condoms consistently are at very high risk for re-infection [19]. Although the rate of transmission of *T. vaginalis* from female to male is still a matter of debate (studies have shown anywhere from 4 to 80%), male clients of FSW must still be considered at high risk to become infected and may act as carriers to other sexual partners [19]. As metronidazole therapy will likely not provide significant protection beyond the duration of treatment, FSW and their clients can act as a community reservoir for *T. vaginalis* and other STI.

There is also often a strong social stigma associated with diagnosis and/or treatment of genital infection. Women in particular may not seek treatment for fear of the stigma disease will cause in their family or community [20]. A study on impoverished, pregnant African women showed that, although none volunteered any symptoms, targeted questioning revealed that 80% had lower abdominal complaints of some type. Of these women, 52% had at least one STI [21]. General knowledge of STI also tends to differ between the sexes, particularly in developing countries where education opportunities for women may be limited. A survey in India showed that women had significantly less understanding of STI transmission, prevention, and treatment than did men. The same survey showed misinformation can be a significant issue. Over two-thirds of men stated their beliefs that mothers who did not breast feed were intentionally harming their babies, were HIV infected, or had been unfaithful to their husbands [20]. Embarrassment, cultural deterrents, and lack of education on STI transmission, prevention, and treatment mean the diseases like trichomoniasis will not be diagnosed and treated appropriately, and persistent cycles of infection and re-infection may occur.

T. vaginalis, other STI, and HIV

While education (e.g. proper condom use) and aggressive treatment have been shown to reduce STI incidence in several studies, globally the number of cases of these infections continues to increase. A number of studies have been undertaken in attempt to define populations at high risk for *T. vaginalis* infection. Most commonly cited risk factors include: an infected partner, low level of education, drug or alcohol abuse, prostitution, a history of STI and/or bacterial vaginosis (BV), and high risk sexual practices (e.g. unprotected sex, multiple partners) [22,23]. These risk factors are often over-lapping and are common to other STI,

so there can be a synergistic effect that increases the overall risk of *T. vaginalis* infection. A longitudinal study examining the risk of acquiring a STI (gonorrhea, chlamydia, or trichomoniasis) found that the strongest positive predictor was the presence of an infection at baseline. Women with a baseline *T. vaginalis* infection were shown to have the greatest risk (16.5%) of developing trichomoniasis again over the one year study period (taking into account possible treatment failure). However, the presence of gonorrhea at baseline also significantly increased the risk of *T. vaginalis* infection (14.8%) when compared to women who had no STI at baseline (2.8% acquired trichomoniasis during the study) [22]. Another study looked at rescreening for trichomoniasis one month after treatment in HIV-negative and -positive women. Women with HIV were over twice as likely to be culture positive for *T. vaginalis* at rescreening (18.3% vs 8% for HIV-negative), and recurrence was more likely to be attributed to re-infection (45% vs 8% for HIV-negative) [24]. Both studies found that re-infection also correlated strongly with unprotected sex with a new partner or multiple partners.

Similar to other STI, infection with *T. vaginalis* has been shown to increase the risk of acquiring HIV. Studies have shown people with trichomoniasis have 1.5—3 times greater risk of seroconvertion when exposed to HIV [23,25]. As *T. vaginalis* is endemic in the same areas of the world as HIV (especially sub-Saharan Africa and south and south-east Asia, which account for 108.5 million or 62.3% of trichomoniasis cases annually) this increased risk suggests that *T. vaginalis* infection be associated with a significant number of people becoming infected with HIV [2]. Additionally, co-infection with *T. vaginalis* and HIV has been show to increase cervical shedding of the virus in women [26] and lead to higher urethral loads of HIV virus and RNA in men [8].

A number of mechanisms have been proposed to account for the increased susceptibility of people with trichomoniasis to HIV. Women with trichomoniasis often have disrupted vaginal flora, as the presence of *T. vaginalis* leads to a reduction or elimination of *Lactobacillus* species bacteria [27]. This causes a rise in the pH of the vagina which in turn activates pH-sensitive trichomonal proteins [3]. The increase in pH may not only promote the growth of *T. vaginalis*, but also create a more favourable environment for HIV as well as the bacteria associated with other STI and BV [27]. Secreted *T. vaginalis* proteins and cell-to-cell contact dependent mechanisms can cause disruption of the vaginal epithelium leading the to development of punctuate mucosal hemorrhages [28]. These lesions enable HIV access to underlying submucosal tissue, allowing the virus to penetrate and spread beyond the genital tract. The protozoan also secretes factors that degrade protease inhibitors which are present in the vagina and prevent HIV from attaching to and entering target cells [29].

T. vaginalis infection leads to a local (vaginal) inflammatory response that is characterized by the recruitment of neutrophils (which are present in the discharge found in symptomatic cases), as well as macrophages and CD4+ (helper) T cells which are susceptible to infection with HIV [30,31]. This inflammatory response also leads to the production of TNF-α which activates CD4+ T cells, increasing their susceptibility to HIV infection. *T. vaginalis*-induced TNF-α has been shown in vitro to significantly increase HIV replication in infected cells [32]. As trichomoniasis not only increases susceptibility to HIV infection, it increases viral replication and shedding in *T. vaginalis*/HIV co-infected individuals, effective control of this parasitic infection could lead to a significant reduction in the global incidence of HIV.

Advantages of a *T. vaginalis* vaccine

High risk sexual behaviors such as lack of condom use, frequent partner change, multiple partners, and engaging in the sex trade represent the most difficult obstacles to overcome in controlling the global burden of trichomoniasis and all STI. High risk individuals are likely to be repeatedly re-infected and ''from a public health perspective ... part of a core group responsible for maintaining disease in the community'' [22]. If prevention is based solely on treatment, these individuals will likely acquire and spread STI even when easily accessible, inexpensive health care is available. Since trichomoniasis is endemic in areas where medical facilities are often few, difficult to access, and unaffordable, simply treating those who have access to a doctor or clinic is not a viable option in controlling *T. vaginalis*.

The development of a vaccine that could provide long-term protection against *T. vaginalis* infection would resolve many of the issues that currently undermine efforts at control and reduce trichomoniasis rates. Education about STI and a healthy sexual lifestyle has been shown to be effective and should continue, but high-risk individuals could be offered vaccination to protect themselves and their partners. Vaccination of women as a part of routine gynecological or pre-natal care would not only reduce infection rates in women, it would prevent trichomoniasis-related

pregnancy complications and the need to treat *T. vaginalis* infection in pregnant women (metronidazole is contraindicated in the first trimester). Establishing a *T. vaginalis* vaccine as part of routine vaccination would also help reduce the social, familial, and community stigma associated with having or seeking treatment for a genital infection.

Vaccination against trichomonad species is already in use commercially. *Tritrichomonas foetus* is a flagellate protozoan similar to *T. vaginalis* that infects cattle. Bovine trichomoniasis is usually asymptomatic in bulls (which facilitates spread of the disease through herds) but can cause vaginal inflammation and discharge in heifers [33] symptoms that mirror those seen in human disease. *Tr. foetus* infection can cause spontaneous abortions in pregnant heifers [33], analogous to *T. vaginalis* infection in women causing premature labour and low birth weight infants. Additionally, in vitro studies using various cell lines have shown that *T. vaginalis* and *Tr. foetus* exhibit similar contact-dependent (adherence) and contact-independent (secreted proteins) mechanisms of cytotoxicity. This cytotoxicity allows the trichomonads to disrupt cell monolayers in vitro, and helps establish and maintain infection in vivo [34,35]. Vaccinating heifers against *Tr. foetus* has been shown to be very efficacious in preventing infection and reducing mortality in fetal calves which can seriously affect the cattle industry (Trich Guard, Fort Dodge Animal Health). Given the similarity between *Tr. foetus* and *T. vaginalis*, development of a vaccine against human trichomoniasis seems both achievable and with the potential for huge financial, social, and health impact globally.

Significant work has already been accomplished indicating that it is possible to develop a vaccine to prevent human trichmoniasis. A small human trial of 100 women with refractory trichomoniasis was conducted in the 1960s. These women were given intravaginal inoculations with increasing numbers of heat-killed *T. vaginalis*. The vaccine was successful therapeutically (100% improvement in clinical symptoms was reported) and appeared to offer some long-term protection. However, the study was never repeated and this method of vaccination has not been pursued, probably due to the cumbrous technique and need for repeated doses [36].

Immune response to *T. vaginalis* infection is heterogeneous. Examination of serum samples from infected women have shown that antibodies were produced to different protein epitopes despite infection by the same trichomonal strain [37]. However some immunogenic epitopes seem to be fairly well conserved across numerous strains. A 115 kDa α-actin protein [38], a number of heat shock proteins [39], and a 100 kDa protein (currently uncharacterized) [37] have been shown to be recognized by sera from a number of different women with trichomoniasis. These common antigenic epitopes mean cross-isolate protection (vaccination with one trichomonal strain protecting against infection with other strains) should be possible. Alternatively a sub-unit vaccine comprised of highly immunogenic epitopes may provide superior protection by eliciting a strong immune response to conserved protein trichomonal proteins.

A mouse model of *T. vaginalis* infection has shown that, as in humans, mice can be infected, cured with metronidazole, and readily re-infected. However, vaccinating mice with *T. vaginalis* (subcutaneous injection of whole cells with adjuvant) provides protection from subsequent vaginal infection, indicating that long-term immunological protection is possible [40]. Vaccination of mice leads to increased production of antibody (IgG) and cytokines, and increased proliferation and maturation of immune cells when compared to vaginally infected animals (unpublished data). The existence of a reliable and relatively inexpensive animal model of *T. vaginalis* infection will aid in elucidating the specific factors required to elicit the strong, sustained, and protective immune response required for an effective vaccine.

Trichomoniasis is the most common non-viral STI in the world, and it is not a ''nuisance'' but a disease that can cause serious consequences to both individual and community health. A *T. vaginalis* vaccine could provide long-term protection instead of short-term cures, reduce medical costs, and prevent sequelae associated with pregnancy and infertility. Preventing *T. vaginalis* infection could have a profound effect not only in reducing the burden of treating infected individuals but on reducing global HIV rates, particularly since the prospect for an HIV vaccine is presently uncertain. The challenge will be to encourage investment in vaccine development where the targeted population is in resource poor communities.

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References

[1] World Bank. World development report: investing in health. Washington: World Bank; 1993.
[2] WHO. Global prevalence and incidence of selected curable sexually transmitted diseases: overview and estimates. Geneva: World Health Organization; 1999.

[3] Petrin D, Delgaty K, Bhatt R, Garber G. Clinical and microbiological aspects of *Trichomonas vaginalis*. Clin Microbiol Rev 1998;11(2):300—17.
[4] Zigas V. An evaluation of trichomoniasis in two ethnic groups in Papua New Guinea. Sex Transm Dis 1977;4: 63—5.
[5] Bowden FJ, Paterson BA, Mein J, Savage J, Fairley CK, Garland SM, et al. Estimating the prevalence of *Trichomonas vaginalis*, *Chlamydia trachomatis*, and *Neisseria gonorroheae* in indigenous women in Northern Australia. Sex Transm Inf 1999;75:431—4.
[6] Wilcox RR. Epidemiological aspects of trichomoniasis. Br J Vener Dis 1960;36:167—74.
[7] Cotch MF, Pastorek JG, Nugent RP, Hillier SL, Gibbs RS, Martin DH, et al. *Trichomonas vaginalis* associated with low birth weight and pre-term delivery. Sex Transm Dis 1997;24:353—60.
[8] Hobbs MM, Kazembe P, Reed AW, Miller WC, Nkata E, Zimba D, et al. *Trichomonas vaginalis* as a cause of urethritis among Malawian men. Sex Transm Dis 1999;26: 381—7.
[9] Saxena SB, Jenkins RR. Prevalence of *Trichomonas vaginalis* in men at high risk for sexually transmitted diseases. Sex Trans Dis 1991;18:138—42.
[10] Weston TE, Nicol CS. Natural history of trichomonal infection in men. Br J Vener Dis 1963;39:251—7.
[11] Lossick J. Treatment of sexually transmitted diseases. Rev Inf Dis 1990;12(Suppl. 6):S665—81.
[12] Cudmore SL, Delgaty KL, Hayward-McClelland SF, Petrin DP, Garber GE. Treatment of infections caused by metronidazole-resistant *Trichomonas vaginalis*. Clin Microbiol Rev 2004;17(4):783—93.
[13] Schmidt G, Narcisi E, Mosure D, Secor WE, Higgins J, Moreno H. Prevalence of metronidazole-resistant *Trichomonas vaginalis* in a gynecology clinic. J Reprod Med 2001;46: 545—9.
[14] Schwebke JR, Barrientes FJ. Prevalence of *Trichomonas vaginalis* isolates with resistance to metronidazole and tinidazole. Antimicrob Agents Chemother 2006;50:4209—10.
[15] Price MA, Stewart SR, Miller WC, Behets F, Dow WH, Martinson FE, et al. The cost-effectiveness of treating male trichomoniasis to avert HIV transmission in men seeking sexually transmitted disease care in Malawi. J Acquir Immune Defic Syndr 2006;43:202—9.
[16] KissingerP, Schmidt N, Mohammed H, Leichliter JS, Gift TL, Meadors B, et al. Patient-delivered partner treatment for *Trichomonas vaginalis* infection: a randomized controlled trial. Sex Transm Dis 2006;33:445—50.
[17] Cwikel JG, Lazer T, Press F, Lazer S. Sexually transmissible infection among female sex workers: an international review with an emphasis on hard-to-access populations. Sexual Health 2008;5:9—16.
[18] Cwikel JG. A textbook of social epidemiology—strategies for social health activism. New York: Columbia University Press; 2006.
[19] Bowden FJ, Garnett GR. *Trichomonas vaginalis* epidemiology: parameterizing and analyzing a model of treatment interventions. Sex Transm Dis 2000;76: 248—56.
[20] Rogers A, Meundi A, Amma A, Rao A, Shetty P, Antony A, et al. HIV-related knowledge, attitudes, perceived benefits, and risks of HIV testing among pregnant women in rural southern India. AIDS Patient Care STDs 2006;20(11): 803—11.
[21] Sturm AW, Wilkinson D, Ndovela N, Bowen S, Connolly C. Pregnant women as a reservoir of undetected sexually transmitted disease in rural South Africa: implications for disease control. Am J Public Health 1998;88: 1243—5.
[22] Peterman TA, Tian LH, Metcalf CA, Satterwhite CL, Malotte CK, DeAugistine N, et al. High incidence of new sexually transmitted infections in the year following a sexually transmitted infection: a case for rescreening. Ann Intern Med 2006;145:564—72.
[23] McClelland RS, Sangare L, Hassan WM, Lavreys L, Mandaliya K, Kairie J, et al. Infection with *Trichomonas vaginalis* increases the risk of HIV-1 acquisition. J Inf Dis 2007;195:698—702.
[24] Kissinger P, Secor WE, Leichliter JS, Clark RA, Schmidt N, Curtin E, et al. Early repeated infections with *Trichomonas vaginalis* among HIV-positive and HIV-negative women. Clin Inf Dis 2008;46:994—9.
[25] Ter Meulen J, Mgaya HN, Chang-Claude J, Luande J, Mtiro H, Mhina M, et al. Risk factors for HIV infection in gynaecological inpatients in Dar es Salaam, Tanazania, 1988—1990. East Afr J 1992;69:688—92.
[26] Kreiss J, Willerford DM, Hensel M, Emonyi W, Plummmer F, Ndinya-Achola J, et al. Association between cervical inflammation and cervical shedding of human immunodeficiency virus DNA. J Inf Dis 1994;170:1597—601.
[27] Moodley P, Connolly C, Sturm AW. Interrelationships between human immunodeficiency virus type 1, bacterial vaginosis, trichomoniasis, and the presence of yeasts. J Inf Dis 2002;185:69—73.
[28] Fouts AC, Kraus SJ. *Trichomonas vaginalis*: reevaluation of its clinical presentation and laboratory diagnosis. J Inf Dis 1980;141:137—43.
[29] Draper D, Donohoe W, Mortimer M, Heine RP. Cysteine proteases of *Trichomonas vaginalis* degrade secretory leukocyte protease inhibitor. J Inf Dis 1998;178: 815—8.
[30] LevineWC, Pope V, Boomhar A, Tambe P, Lewis JS, Zaidi AA, et al. Increase in endocervical CD4 lymphocytes among women with nonulcerative sexually transmitted diseases. J Inf Dis 1998;177:167—74.
[31] Sardana S, Sodhani P, Agarwal SS, Sehgal A, Roy M, Singh V. Epidemiologic analysis of *Trichomonas vaginalis* infection in inflammatory smears. Acta Cytol 1994;38: 693—7.
[32] Guenthner PC, Secor WE, Dezzutti CS. *Trichomonas vaginalis*-induced epithelial monolayer disruption and human immunodeficiency virus type 1 (HIV-1) replication: implications for the sexual transmission of HIV-1. Inf Immun 2005;73(7):4155—60.
[33] BonDurant RH, Honigberg BM. Trichomonads of veterinary importance. Parasitic Protozoa, vol. 9. New York, NY: Academic Press; 1994. p. 111—188.
[34] Silva Filho FC, de Souza W. The interaction of *Trichomonas vaginalis* and *Tritrichomonas foetus* with epithelial cells in vitro. Cell Struct Funct 1988;13:301—10.
[35] Burgess DE, Knoblock KF, Daugherty T, Robertson NP. Cytotoxic and hemolytic effects of *Tritrichomonas foetus* on mammalian cells. Infect Immun 1990;58(11): 3827—32.
[36] Aburel E, Zervos G, Titea V, Pana S. Immunological and clinical investigations into vaginal trichomoniasis. Rum Med Rev 1963;7:13—9.
[37] Garber GE, Proctor EM, Bowie WR. Immunogenic proteins of *Trichomonas vaginalis* as demonstrated by the immunoblot technique. Infect Immun 1986;51:253—63.
[38] Addis MA, Rappelli P, de Andrade AMP, Rita FM, Colombo M, Capuccinelli P, et al. Identification of *Trichomonas vaginalis* α-actinin as the most common antigen recognized by

the sera of women exposed to the parasite. J Infect Dis 1999;180:1727—30.
[39] Davis-Hayman SR, Shah PH, Finley RW, Meade JC, Lushbaugh WB. Antigenicity of *Trichomonas vaginalis* heat-shock proteins in human infections. Parasitol Res 2000;86:115—20.
[40] Hayward-McClelland SF, Delgaty KL, Garber GE. Animal models of *Trichomonas vaginalis* infection with special emphasis on the intravaginal mouse model. In: Handbook of animal models of infection. New York, NY: Academic Press; 1999. p. 839—50.