Bilateral Conjunctivitis Due to *Trichomonas vaginalis* without Genital Infection: an Unusual Presentation in an Adult Man

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We report an unusual case of extragenital infection with *Trichomonas vaginalis* of the conjunctiva of a 32-year-old man. Only one other similar case has been reported in the English language literature. The present report reinforces the widening pathologic spectrum of trichomonads in humans, especially in the context of emerging extragenital infections.

CASE REPORT

A 32-year-old Iranian man presented to a private infectious disease clinic with a 2-week history of inflamed conjunctivae and a yellowish, purulent discharge emanating from both eyes. He was previously prescribed topical 1% chloramphenicol ointment by another clinician a week after the onset of his symptoms with no obvious improvement. On physical examination, he appeared well and his vision was normal. A diagnosis of bacterial conjunctivitis was made, and the patient was started on oral flucloxacillin at 250 mg every 6 h to cover the staphylococcal infection often associated with conjunctivitis and topical 0.3% ciprofloxacin and 0.5% chloramphenicol eye drops.

Before starting treatment, multiple conjunctival swabs were collected from the lower conjunctiva with flexible, fine, plastic-shafted Dacron swabs premoistened with sterile saline for microbiological investigation, including bacterial and viral cultures and microscopy in accordance with routine practice. For bacterial culture, swabs were directly inoculated into Columbia blood agar, chocolate agar, and MacConkey agar, where only scanty normal skin flora was isolated after 48 h of incubation and later identified as *Staphylococcus epidermidis*. Viral cultures for adenoviruses, herpes simplex viruses 1 and 2, and enteroviruses were all negative. Results of direct immunofluorescence microscopy and culture for *Chlamydia trachomatis* were negative.

Interestingly, microscopic examination of a Gram-stained smear revealed moderate numbers of nondescript cell-like structures that were indistinguishable mixed with numerous white blood cells, predominantly polymorphonuclear leukocytes. In an attempt to identify the cell-like structures seen on the Gram-stained smear, a second Giemsa-stained smear was examined on the same day. On the basis of the morphological features of the cells, namely, an ameboid shape, the presence of one or two elliptically shaped nuclei, and a poorly defined cytoplasm, a provisional identification of trichomonad parasites was made. Two original separate eye swabs (left and right) were retrieved roughly 5 h postcollection and immediately inoculated into Kupferberg *Trichomonas* culture medium (QUELAB) for further analysis. This medium was supplemented with heat-inactivated bovine serum, penicillin G (10,000 U/ml), streptomycin (10,000 µg/ml), and amphotericin B (25 µg/ml). Cultures were incubated at 37°C in the presence of 5 to 7% CO₂ and examined microscopically on days 2, 5, and 7. Subsequently, two cultures became positive for mobile trophozoites of trichomonads.

At this stage, considering the isolation of the trichomonads from both eyes, the patient was recalled to the clinic. When he arrived, two new conjunctival swabs were collected as previously described to exclude environmental and/or cross contamination of the original samples. Furthermore, to exclude autoinoculation of a parasite from the patient’s urethra to his eye, one urethral swab was also obtained. All three swabs were cultured for trichomonad parasites as described earlier. At this point, as our patient’s conjunctivitis was still present, metronidazole (800 mg three times daily for 5 days) was added to his treatment. In addition, he was asked to return for follow-up a week after the completion of his recent treatment. On the next visit, all of his symptoms were completely resolved and both eyes showed satisfactory resolution. The test-of-cure result was negative.

In order to identify the trichomonad species isolated in the culture medium, a PCR strategy was used. Parasites were washed two times in sterile phosphate-buffered saline (pH 7.2) and subjected to DNA extraction. The pellet was suspended in 400 µl Tris-EDTA buffer. DNA extraction was performed with SDS and proteinase K, following by cetyltrimethylammonium bromide-NaCl. The presence of DNA in each sample was confirmed by electrophoresis prior to PCR amplification.

For detection of *Trichomonas vaginalis*, oligonucleotide primers TVK3 (5’TATTGTGCAACATGGTCTTACCTC-3’) and TVK7 (5’-TCTGTGCCGTCTTCAATGGTCG-3’) were used (TIB MOLBIOL). The primers specifically amplify a target within a 2,000-bp repeat region of the *T. vaginalis* genome, producing a 300-bp amplicon (1). PCRs were performed with a programmed thermocycler (Eppendorf Mastercycler gradient). A standard PCR was carried out with a total volume of 50 µl. The final reaction mixture contained 25 µl of HotStarTaq master mix, 1 µl of 10 mM

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TVK3, 1 µl of 10 mM TVK7, 13 µl of RNase-free distilled water, and 10 µl of prepared DNA. HotStarTaq master mix contained 2.5 U HotStarTaq DNA polymerase, 1× PCR buffer (15 mM MgCl₂), and 200 µM each dextranucleotide triphosphate (Qiagen). The PCR protocol consisted of an initial 15 min of incubation at 95°C, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 30 s, and extension at 72°C for 2 min. Amplification finished with a heating step at 72°C for 2 min for completion of the extension phase. A DNA extract from *T. vaginalis* ATCC 50143Y was used as a positive control. The negative control consisted of PCR master mix with distilled water. PCR products from cultures were analyzed by gel electrophoresis (GE). Eight microliters of PCR product was mixed with 2 µl of loading buffer and then separated by horizontal GE at 75 V for 1 h on a 1.5% agarose gel (pH 8.5). The gel was stained with ethidium bromide (0.5 µg/ml) and visualized and photographed with a UV light illuminator. The size of the amplicon was measured by comparison with a commercial 100-bp DNA ladder (Fig. 1). Cultures from both the left and right eyes containing a 300-bp fragment were considered positive for *T. vaginalis*.

*T. vaginalis* is a protozoan parasite that infects the urogenital tracts of men and women, causing trichomoniases with a worldwide presence and significant implications for global public health. An estimated 150 million new cases of trichomoniases occur worldwide each year, making it the most common nonviral sexually transmitted disease in the world (2). Until recently, trichomoniase parasites of humans were thought to be site specific. *T. vaginalis* is considered the only pathogenic trichomonad and causes infection mainly of the genitourinary tract. However, the presence of this protozoan parasite in the human respiratory tract has been reported previously (3–5). In addition, with more sensitive molecular methods, other trichomonads, namely, *Trichomonas tenax*, *Pentatrichomonas hominis*, *Tri Trichomonas foetus*, *Tetratrichomonas gallinarum*, and a new *Tetratrichomonas* species, have been found in a number of cases in the respiratory tracts of humans (6, 7).

Extragenital trichomoniases is a very rare event. In the case reported here, we unexpectedly identified *T. vaginalis* trophozoites in eye swab samples from an adult man with conjunctivitis, which highlights the increasing interest in trichomonad parasites. To our knowledge, this is the second case of *T. vaginalis* infection of the conjunctiva and the first in an adult patient. The first report was a case of bilateral conjunctivitis in a 17-day-old male with a yellowish and purulent discharge. Concomitant infection with *Staphylococcus aureus* was present, and trichomoniases were observed only in a Papanicolaou-stained smear examined microscopically; hence, differentiation between *T. vaginalis* and other trichomoniases was not achieved. However, the genital tract of the mother was suggested as the source of infection (8). Nevertheless, in another study, conjunctival trichomoniases could not be demonstrated in 272 newborns despite evidence of trichomoniases in 3.7% of the pregnant mothers (9).

Because of the unusual infection site and in order to explain the route of transmission in our patient, an in-depth, face-to-face interview was conducted. After more direct and detailed questioning, the patient had admitted that his eyes had been contaminated with his recent female partner’s genital secretions during his last sexual activity. Although it was not possible to contact his sex partner, we believe that in the absence of any trichomoniases in our patient’s urethral samples, direct contact between his female partner’s genitalia and his eyes did facilitate the transmission of parasites, which led, in turn, to his conjunctivitis. It is less likely that infected genital secretions were transferred to our patient’s eyes by his contaminated hands.

Urogenital trichomoniases is transmitted almost exclusively by sexual intercourse. Although factors affecting the transmission or concordance of *T. vaginalis* infection between sexual partners are largely unknown, direct physical contact facilitates parasite adherence to and penetration of the mucosal surfaces, which subsequently elicits a host inflammatory response. Similarly, a clear direct and physical contact pathway is most likely required for parasite transmission to extragenital sites. As was shown in our case, mechanical exposure of healthy conjunctivae to vaginal secretions harboring *T. vaginalis* parasites led to conjunctival infection in an adult man. In addition, rare cases of respiratory tract infections in infants born to mothers with histories of genital trichomoniases indicate the same route of transmission (4, 5). The recent molecular detection of *T. vaginalis* in pharyngeal specimens from three HIV-infected men with histories of orogenital sexual activity (10) highlights the need for further research to unravel the transmission dynamics and pathogenesis of extragenital trichomoniases.

Indeed, the lack of reports in the medical literature may not reflect the true incidence of extragenital infections due to *T. vaginalis*. The use of molecular methods for the detection of trichomoniases in clinical samples and identification to the species level may widen our understanding of the incidence and pathogenicity of these protozoan parasites several decades after their initial discovery.

REFERENCES