CASE REPORTS

Molecular Identification of *Tritrichomonas foetus*-Like Organisms as Coinfecting Agents of Human *Pneumocystis* Pneumonia

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Trichomonads closely related to the bovid parasite *Tritrichomonas foetus* were identified in the bronchoalveolar lavage sample from a patient with AIDS in association with *Pneumocystis* pneumonia. This human case of *T. foetus*-like infection emphasizes the zoonotic potential of trichomonads, although the existence of a human-host-adapted *T. foetus* strain cannot be excluded.

CASE REPORT

A 54-year-old woman was admitted to the intensive care unit of Mantes-la-Jolie hospital, presenting with fever, severe fatigue, and dyspnea with polypnea that had been increasing over the previous month. The patient was known to be seropositive for human immunodeficiency virus, which was revealed 3 years previously following pneumocystosis. At that time, an antiretroviral therapy was proposed but the patient denied the diagnosis. Since the patient had no history of blood transfusion or injection of illicit drugs, transmission by unprotected sex remained the presumed mode of human immunodeficiency virus contamination. In addition, the patient was overweight and had type 2 diabetes. Hemodynamic parameters were within normal limits. Blood gases were 52 mm Hg O_2 and 36 mm Hg CO_2. A chest X-ray showed bilateral interstitial syndrome and several alveolar opacities. Bronchoalveolar lavage (BAL) was performed by fiber optic bronchoscopy and confirmed pneumocystosis. Interestingly, microscopic examination of a May-Grünwald-Giemsa-stained cytospin slide revealed numerous cells assumed to be trichomonad organisms, mixed with *Pneumocystis* parasites. The BAL sample was therefore immediately frozen for further analysis. Nasal oxygenation, intravenous injection of trimethoprim-sulfamethoxazole and methylprednisolone hemisuccinate, and subcutaneous injection of insulin were started, and recovery was rapid. On day 4, the patient was transferred to the Department of Infectious Diseases of the same institution where the investigations were completed. The CD4 cell count was 60 per mm³, with a CD4/CD8 ratio of 0.06.

Plasma viral load was 5.34 $\log_{10}$ copies/ml. Hypercalcemia with hyperparathyroidism was also noticed. The patient returned home 13 days after her hospitalization with trimethoprim-sulfamethoxazole per os. Antiretroviral therapy and exploration of hyperparathyroidism were scheduled.

One part of the BAL sample obtained by fiber optic bronchoscopy was centrifuged, and cytospin slides were obtained for subsequent standard microscopic examination. The BAL specimen examined contained numerous parasites provisionally identified as trichomonads, exhibiting an amoeboid shape and mixed with alveolar macrophages, lymphocytes, erythrocytes, and aggregates of *Pneumocystis* organisms (Fig. 1). Parasitic cells showed one or two elliptically shaped nuclei and a poorly defined cytoplasm. This appearance had already been described for trichomonads from cytological specimens stained with May-Grünwald-Giemsa or Papanicolaou techniques (1–5). In parallel, DNA was extracted from the remaining part of the BAL sample by use of a QIAamp DNA minikit (QIAGEN, Hilden, Germany), with some modifications to the manufacturer's recommendations. In order to identify the trichomonad species found in the BAL sample, a nested-PCR strategy was developed, allowing the DNA amplification of the internal transcribed spacer 1 (ITS1)-5.8S rRNA-ITS2 region by use of trichomonad-specific primers. The first PCR was performed using the sense primer TRICHO-F (5′-CGGTAGGTGAACC TGCCGTT-3′) and the antisense primer TRICHO-R (5′-TG CTTCAGTTCAGCGGGTCT-3′) as described previously (8). PCR was carried out for 40 cycles (GeneAmp PCR system 9700 apparatus; Applied Biosystems) according to standard conditions for Platinum *Taq* high-fidelity DNA polymerase (Invitrogen, Groningen, The Netherlands). Negative (BAL sample without trichomonads) and positive (trichomonad DNAs extracted from axenic cultures) controls were included

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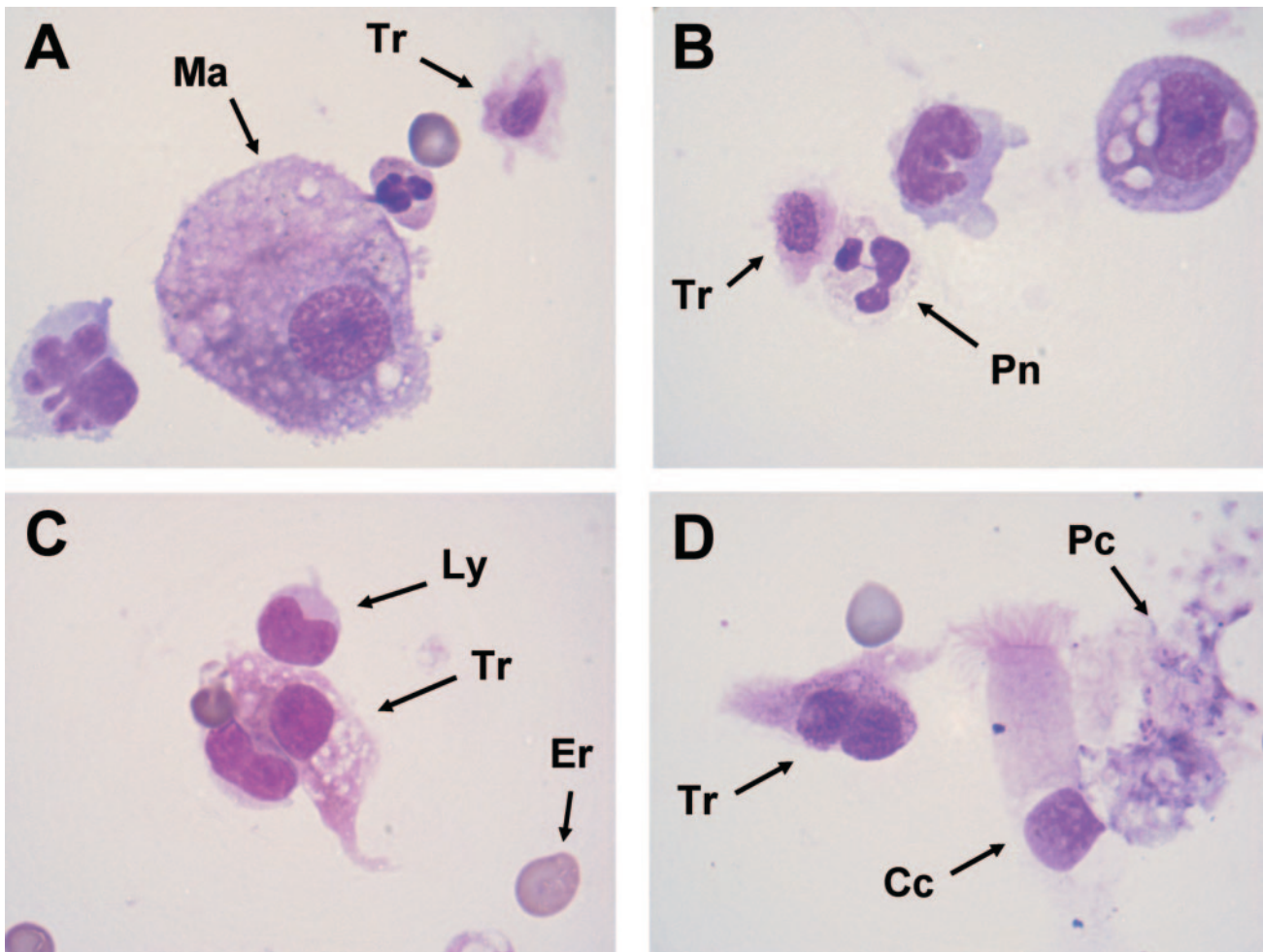
FIG. 1. Cytological appearance of trichomonad cells in the BAL sample (May-Grünwald-Giemsa staining). (A) A trichomonad cell (Tr) with an oval nucleus is easily recognizable in the vicinity of a macrophage cell (Ma). (B) A trichomonad cell is seen in contact with a neutrophile polymorphonuclear (Pn). (C) An amoeboid trichomonad with a round nucleus is seen between two lymphocytes (Ly); an erythrocyte (Er) gives the scale (diameter, 7 μm). (D) An amoeboid trichomonad exhibiting two nuclei is seen in the vicinity of a bronchial ciliated cell (Cc) and an aggregate of *Pneumocystis* organisms (Pc). Magnification, ×1,000.

in the series. The second amplification was performed using the sense primer TRICHO-FBIS (5′-GGTGAACCTGCCGT TGGATC-3′) and the antisense primer TRICHO-RBIS (5′-T CAgTTCAGCGGGTCTTCCT-3′). The second PCR product was separated by agarose gel electrophoresis, and the band of the expected size (323 bp excluding the amplification primers) was purified using a QIAEX II gel extraction kit (QIAGEN). The purified PCR product was cloned in the T vector pCR 2.1-TOPO (Invitrogen) and amplified in *Escherichia coli* TOP10 competent cells. Ten clones were arbitrarily isolated, and the resulting minipreparations of plasmid DNAs were done using a QIAprep spin miniprep kit (QIAGEN). Selected clones were sequenced on both strands by use of a Big Dye Terminator cycle sequencing kit (Applied Biosystems) and an automated PRISM 377 DNA sequencer (Applied Biosystems).

Among the 10 sequenced clones, 8 of them (clones 1 to 8) exhibited 100% identity, whereas clones 9 and 10 differed from clones 1 to 8 at only one position (C→T and T→C in positions 138 and 203, respectively; numbering as for the 323-bp PCR product). These differences are likely to be due to normal

variation within the multiple copies of the RNA genes in any given genome. These sequences were aligned with all of the trichomonad ITS1-5.8S-ITS2 sequences available in databases by use of the BioEdit v7.0.1 package (http://www.mbio.ncsu .edu/BioEdit/bioedit.html). A part of this alignment, including only trichomonad species of interest in our study, is shown in Fig. 2. In the common part of our alignment (339 positions including gaps), the sequences of clones 1 to 10 showed a lower degree of similarity (57.9 to 64.3%) to sequences of other trichomonad species found in humans. These included *Trichomonas vaginalis*, *Trichomonas tenax*, *Pentatrichomonas hominis*, and the recently identified *Tetratrichomonas* sp. strains TXB3 and TXO6, isolated from the oral cavity and bronchi of patients, respectively (9). Strikingly, clones 1 to 10 exhibited 98.6 to 99.2% identity (3 to 5 nucleotide differences) to homologous sequences from *Tritrichomonas foetus*, *Tritrichomonas suis*, and *Tritrichomonas mobilensis*, three probably synonymous trichomonad species isolated from cattle, pigs, and squirrel monkeys, respectively (6, 9, 12, 19). These results showed that the trichomonad species found in the lungs of this patient belonged to the *Tritrichomonas* genus

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Trichomonas vaginalis Tv1:MCP L29561    CGGTAGGTGAACCTGCCGTTGGATCAGTTCTAGTT*TTAATAACTAACACCAA*CTTC~TTTTATT**AAACAAAAACCAATACAAAATT  85
Trichomonas tenax 30207 U86615                         TRICHO-F      ATCAGTTCTAGTT**TAATAACTAATACCAA*CTTCTTTTTATT**AATCAAAAACTAATACAATTAT  63
Tetratrichomonas sp. TXB3 AY244641                                   ATCAGTTCTAGTT*TTAATAACTAACACCAA*CTTCTTTTTAATTAA*TTTTTAAAACCACATA**CAA  63
Tetratrichomonas sp. TXO6 AY244644                                   ATCAGTTCTAGTT**TAATAACTAACACCAA*CTTCTTTTTAATTAACTTTTTAAAACCACATA**CAA  64
Pentatrichomonas hominis 30098 U86616                                ATCAGTTCTAGTT*********TAACACCAAACTTCTTTTT**T**GAT**AAAACCAATACAAT*AT  52
Tritrichomonas mobilensis 50116 U86612  CGGTAGGTGAACCTGCCGTTGGATCAGTT*TCGTTAATAATTACAAACATATTTTTTAATGTCTA**TAACTATTT***ATACAAAATT  84
Tritrichomonas foetus 30233 M81842      CGGTAGGTGAACCTGCCGTTGGATCAGTT*TCGTTAATAATTACAAACATATTTTTTAATGTCTA**TAACTATTT***ATACAAAATT  84
Tritrichomonas suis U85966              CGGTAGGTGAACCTGCCGTTGGATCAGTT*TCGTTAATAATTACAAACATATTTTTTAATGTCTA**TAACTATTT***ATACAAAATT  84
Clones 1 to 8                                        TRICHO-FBIS      AGTT*TCGTTAATAATTACAAACATATTTTTTAATTTCTA**TAACTATTT***ATACAAAATT  59
Clone 9                                                              AGTT*TCGTTAATAATTACAAACATATTTTTTAATTTCTA**TAACTATTT***ATACAAAATT  59
Clone 10                                                             AGTT*TCGTTAATAATTACAAACATATTTTTTAATTTCTA**TAACTATTT***ATACAAAATT  59
                                        -----------SSU rRNA----------|--------------------------------ITS1----------------------

Trichomonas vaginalis Tv1:MCP L29561    AAA*AACTAACTTCATCAAAAA**CCAAGTCTCTAAGCAATGGATGTCTTGGCTCCTCACACGATGAAGAACGTGGCATAATGTGTTAAG 171
Trichomonas tenax 30207 U86615          AAATAACTAACTTCATCAAAAA**TCAAGTCTCTAAGCAATGGATGTCTTGGCTCCTCACACGATGAAGAACGTGGCATAATGTGATAAG 150
Tetratrichomonas sp. TXB3 AY244641      CAACAAAAAACATAATAAAAAA*GTTAGGACTCTAATCAACGGATGTCTTGGCTCCTTACACGATGAAGAACGTTGCATAATGTGATAAG 151
Tetratrichomonas sp. TXO6 AY244644      CAAACAAAAAAATAATAAAAAAAGTTAGGACTCTAATCAACGGATGTCTTGGCTCCTTACACGATGAAGAACGTTGCATAATGTGATAAG 153
Pentatrichomonas hominis 30098 U86616   AAAC**CTAACTT*****AATG**TAAGGTCTCTAAGCAATGGATGTCTTGGCTCCTCACACGATGAAGAACGTTGCATAATGTGATAAG 132
Tritrichomonas mobilensis 50116 U86612  AAACACATAATCTAA***AAAA**TTTAGACCTTAGGCAATGGATGTCTTGGCTTCTTACACGATGAAGAACGTTGCATAATGCGATAAG 168
Tritrichomonas foetus 30233 M81842      AAACACATAATCTAA***AAAA**TTTAGACCTTAGGCAATGGATGTCTTGGCTTCTTACACGATGAAGAACGTTGCATAATGCGATAAG 168
Tritrichomonas suis U85966              AAACACATAATCTAA***AAAA**TTTAGACCTTAGGCAATGGATGTCTTGGCTTCTTACACGATGAAGAACGTTGCATAATGCGATAAG 168
Clones 1 to 8                           AAACACATAATCTAA***AAAA**TTTAGACCTTAGGCAATGGATGTCTTGGCTTCTTACACGATGAAGAACGTTGCATAATGCGATAAG 143
Clone 9                                 AAACACATAATCTAA***AAAA**TTTAGACCTTAGGCAATGGATGTCTTGGCTTCTTACACGATGAAGAACGTTGCATAATGCGATAAG 143
Clone 10                                AAACACATAATCTAA***AAAA**TTTAGACCTTAGGCAATGGATGTCTTGGCTTCTTACACGATGAAGAACGTTGCATAATGCGATAAG 143
                                        -------------------------------|--------------------5.8S rRNA---------------------

Trichomonas vaginalis Tv1:MCP L29561    TAACCGGAGTTGCAAACATCATGACAGGTT*AATCTTTGAATGCAAATTGCGCTAAACTCGATC*TCGGTCG*AGAA**GCATGGGTGTG 256
Trichomonas tenax 30207 U86615          TAACCGGAGTTGCATACATCATGACAGGTT*AATCTTTGAATGCAAATTGCGCTAAACTTGGCT*TCGGCTG*AGAA**GCATGCGTGTA 235
Tetratrichomonas sp. TXB3 AY244641      TAACTGGAGTTGCGAACATTGTGACAAGTT*AATCTTTGAATGCAAATTGCGCGTACCTTTGCTCTCGGGCA*TTGGTTGCATGTATGCC 239
Tetratrichomonas sp. TXO6 AY244644      TAACTGGAGTTGCGAACATTGTGACAAGTT*AATCTTTGAATGCAAATTGCGCGTATCTTTGCTCTCGGGCA*TTGATTGCATGTATGCC 241
Pentatrichomonas hominis 30098 U86616   TAACTGGAGTTGCGAACATTGTGACAAGTT*AATCTTTGAATGCAAATTGCGCATACCTGAATT*T*ATTC**AGAT**GCATATATATT 215
Tritrichomonas mobilensis 50116 U86612  CGGCTGGATTAGCTTTCTTTGCGACAAGTTCGATCTTTGAATGCACATTGCGCGCCGTTTAGC*TTGCTAGAACAC**GCATATATGTT 255
Tritrichomonas foetus 30233 M81842      CGGCTGGATTAGCTTTCTTTGCGACAAGTTCGATCTTTGAATGCACATTGCGCGCCGTTTTAGC*TTGCTAGAACAC**GCATATATGTT 255
Tritrichomonas suis U85966              CGGCTGGATTAGCTTTCTTTGCGACAAGTTCGATCTTTGAATGCACATTGCGCGCCGTTTTAGC*TTGCTAGAACAC**GCATATATGTT 255
Clones 1 to 8                           CGGCTGGATTAGCTTTCTTTGCGACAAGTTCGATCTTTGAATGCACATTGCGCGCCGTTTAGC*TTGCTAGAACAC**GCATATATGTT 230
Clone 9                                 CGGCTGGATTAGCTTTCTTTGCGACAAGTTCGATCTTTGAATGCACATTGCGCGCCGTTTTAGC*TTGCTAGAACAC**GCATATATGTT 230
Clone 10                                CGGCTGGATTAGCTTTCTTTGCGACAAGTTCGATCTTTGAATGCACATTGCGCGCCGTGTTAGC*TTGCTAGAACAC**GCATATATGTT 230
                                        -------------------------------------------------------5.8S rRNA------------------------

Trichomonas vaginalis Tv1:MCP L29561    ACAGTACTACATCTTT*TATAAT****AATTCTTATTCTAAGCGAATAAGTAAATAATATATAAG*ACAAACAACACGTAGTCTGCCATA 340
Trichomonas tenax 30207 U86615          ACAGTACAACATAATT*TATAT****AATTCTTATTCTAAGCGAATAAGTAAATAAATTATAAG*ACAAACTT*ACGTTGTCTGCTATA 318
Tetratrichomonas sp. TXB3 AY244641      ACAGTACTGCATAAAA*TAATAT****ATATTCTTCTTTTCGCAAAGAAGCAAATATTATTTAAGCAAAAATGT*ACGTAGTCTGCGACA 323
Tetratrichomonas sp. TXO6 AY244644      ACAGTACTGCATAAAA*TAATAT****ATATTCTTCTTTTCGCAAAGAAGCAAATATTATTTAAGCAAAAATGT*ACGTAGTCTGCGACA 325
Pentatrichomonas hominis 30098 U86616   CTAGTACTACATCTTT***TAAT****AATTCTTCTTATAA***AAGAAGCAAATAACT*AAAAG**CAAACA***CGTAGTCTGGGATA 289
Tritrichomonas mobilensis 50116 U86612  ACAGTAACCCATATTAATTCAATACCAAATTCTCTTTTTAAGCAAAGAGCGAAAAACAAATATGTATTAACAAAA*GGGTTCTGTCTCA 344
Tritrichomonas foetus 30233 M81842      ACAGTAACCCATATTAATTTAATACCAAATTCTCTTTTTAAGCAAAGAGCGAAAAACAAATATGTATTAACAAAA*GGGTTCTGTCTCA 344
Tritrichomonas suis U85966              ACAGTAACCCATATTAATTTAATACCAAATTCTCTTTTTAAGCAAAGAGCGAAAAACAAATATGTATTAACAAAA*GGGTTCTGTCTCA 344
Clones 1 to 8                           ACAGTAACCCATATTAATTTAACGCCAAATTCTCTTTTTAAGCAAAGAGCGAAAAACAAATATGTATTAACAAAA*GGGTTCTGTCTCA 319
Clone 9                                 ACAGTAACCCATATTAATTTAACGCCAAATTCTCTTTTTAAGCAAAGAGCGAAAAACAAATATGTATTAACAAAA*GGGTTCTGTCTCA 319
Clone 10                                ACAGTAACCCATATTAATTTAACGCCAAATTCTCTTTTTAAGCAAAGAGCGAAAAACAAATATGTATTAACAAAA*GGGTTCTGTCTCA 319
                                        -----------|--------------------------------ITS2-----------------------|---------------

Trichomonas vaginalis Tv1:MCP L29561    CGCAGGAAGACCCGCTGAACTGAAGCA 368
Trichomonas tenax 30207 U86615          CGCA          TRICHO-R          323
Tetratrichomonas sp. TXB3 AY244641      TACA                            328
Tetratrichomonas sp. TXO6 AY244644      TACA                            330
Pentatrichomonas hominis 30098 U86616   TGTA                            294
Tritrichomonas mobilensis 50116 U86612  TATAGGAAGACCCGCTGAACTGAAGCA 372
Tritrichomonas foetus 30233 M81842      TATAGGAAGACCCGCTGAACTGAAGCA 372
Tritrichomonas suis U85966              TATAGGAAGACCCGCTGAACTGAAGCA 372
Clones 1 to 8                           TAT           TRICHO-RBis        323
Clone 9                                 TAT                             323
Clone 10                                TAT                             323
                                        ----------LSU rRNA---------
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FIG. 2. Alignment of sequences of the ITS1-5.8S rRNA-ITS2 region of clones 1 to 10 obtained in this study and those of trichomonad species of interest found in humans and animals. Differences between the clones obtained in this study and differences between these clones and the homologous sequences of *Tritrichomonas foetus*, *Tritrichomonas suis*, and *Tritrichomonas mobilensis* are shaded. Sequences of the primers used in this study are underlined. Gaps are represented by asterisks. SSU, small subunit; LSU, large subunit.

and indicated that these organisms were closely related to the parasites of bovids, pigs, and nonhuman primates.

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Trichomonads are flagellated protozoa often found in the digestive or reproductive systems of both invertebrates and vertebrates, including mammals. Four trichomonad species are currently found in humans (7): *Trichomonas tenax* in the oral cavity, *Pentatrichomonas hominis* and *Dientamoeba fragilis* in the intestinal tract, and *Trichomonas vaginalis* in the genitourinary tract. Only *T. vaginalis* is considered pathogenic and is the causative agent of human trichomoniasis, which has emerged as the most prevalent nonviral sexually transmitted disease

worldwide in recent years (17). Until recently, trichomonad parasites of humans were thought to be site specific. However, trichomonads identified as *T. tenax* have been found in numerous cases in the upper or lower respiratory tract of humans (11, 13, 14). This organism is a commensal of the human oral cavity and is believed to enter the respiratory tract by aspiration from the contaminated oropharynx. Interestingly, other trichomonad species have also been identified in human lungs by molecular means. These include *T. vaginalis* (4, 15, 18), *P. hominis* (8), and a new *Tetratrichomonas* species that had not previously been reported as a human parasite (9). Thus, the presence of these protozoa in the human respiratory tract is not unusual, and we have recently shown that trichomonads are frequently

found in lungs of immunocompromised patients in association with *Pneumocystis* organisms (5). Pulmonary trichomoniases have been reported almost exclusively with patients with underlying respiratory diseases and/or immunodepression, and trichomonads could not persist, apparently, without associated bacterial or fungal infections. These observations suggested that these protozoa were unable to cause the pulmonary disease on their own. However, their presence should not be considered a benign finding until the pathogenic potential of these parasites has been resolved. In addition, their occurrence in the respiratory tract of humans is likely overlooked, due mainly to observers' lack of awareness in unusual sites and amoeboid transformation of these microorganisms (4, 5).

Recently, the amplification of the ITS1-5.8S rRNA-ITS2 region by PCR followed by sequencing has become a reliable tool for detection and identification of trichomonads at the species level. In the case reported here, we have unexpectedly identified *T. foetus*-like organisms in the BAL sample from an adult patient with AIDS, which reinforces the increasing interest in trichomonad parasites. To our knowledge, this is the second human case of *T. foetus*-like infection, the first one being represented by a case of *T. foetus* meningoencephalitis in a recipient of allogeneic peripheral blood stem cell transplantation (16). However, in the latter case, these microorganisms were identified by scanning electron microscopy examination and not by using molecular tools. Trichomonads were thought to have strict host specificity, and *T. foetus* is associated in nature only with bovine diseases such as vaginitis. However, it has been shown recently that this parasite is also the etiological agent of feline trichomonal diarrhea (10). In addition, molecular data have confirmed previous morphological observations suggesting that *T. foetus*, *Tritrichomonas suis* from pigs, and probably *Tritrichomonas mobilensis* isolated from nonhuman primates should be considered strains of the same species (6, 9, 12, 19). To prevent confusion, it has been proposed to maintain the name *T. foetus* at least for both bovid and porcine *Tritrichomonas* (19). The suggestion that the same *T. foetus* species could be able to colonize several hosts, including bovids, pigs, felids, nonhuman primates, and humans (this study), raises the question of the as-yet-unknown zoonotic potential of trichomonads. These data also suggest the existence of a large potential reservoir in animals for *T. foetus*-like infections in humans. Kutisova et al. (9) have identified *Tetratrichomonas* strains isolated from the respiratory tract of humans which exhibited a very close genetic relationship with a common avian species, *Tetratrichomonas gallinarum*, reinforcing the hypothesis of zoonotic human infections. However, the same authors failed to transmit *Tetratrichomonas* of human origin to birds, which could be explained by a biological separation of the human-host-adapted *T. gallinarum*-like trichomonads. Thus, the existence of a human-host-adapted *T. foetus*-like strain cannot be excluded and this hypothesis has to be tested in further experimental infections. In parallel, additional studies are required to clarify how frequent human infections by *T. foetus*-like organisms are and to determine the pathogenic potential of these parasites. From a more general point of view, the present report highlights the widening pathological spectrum of trichomonads in humans, especially in the context of immunodepression-related emerging infections.

Nucleotide sequence accession numbers. The nucleotide sequences obtained in this study have been deposited in GenBank under accession numbers DQ243910 to DQ243912.

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