Trichomonas tenax detected in pleural effusion fluid: A case report

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Received July 24, 2024; Accepted October 25, 2024

DOI: 10.3892/br.2024.1909

Abstract. *Trichomonas tenax* is predominant in dental caries and is commonly observed in patients with oral diseases; however, its presence in patients with pleural effusion remains rare. Pleural effusion can arise from various causes, including malignant tumors, tuberculosis and bacterial infections. Concurrent infections involving bacteria, fungi and *Trichomonas* are infrequent. This scenario is particularly rare in patients with tumor-associated *Trichomonas tenax* infection. The current study presents a case of *Trichomonas tenax* infection in a patient with a lung tumor. The patient, a 71-year-old male, experienced symptoms of chest tightness, shortness of breath, coughing and expectoration following surgery for a right lung tumor. The expectorated sputum was white and sticky, making coughing difficult. The patient had a history of a prior right lung tumor resection and was subsequently admitted to Heping Hospital Affiliated to Changzhi Medical College (Changzhi, China). Routine examination of the pleural effusion fluid revealed the presence of *Trichomonas tenax* under a wet-film microscope. Molecular sequencing confirmed that the isolate was *Trichomonas tenax*. This case highlights *Trichomonas tenax* as a potential opportunistic pathogen in patients with lung cancer, underscoring the need for heightened clinical awareness. This study offers valuable insights for the diagnosis and prevention of infectious diseases among patients with cancer in the future.

Introduction

Parasites play significant roles in human diseases worldwide. The clinical severity and outcomes of parasitic diseases typically hinge on the immune status of the host (1). As society evolves and lifestyles and dietary patterns change, opportunistic parasitic diseases have increased among individuals with compromised immune systems, along with an increase in imported parasitic diseases (2). This trend is particularly pronounced among cancer patients, whose primary treatment modalities, namely surgery, radiotherapy and chemotherapy, can weaken immune function and predispose them to pathogenic infections (3).

There are three types of trichomonads that parasitize the human body based on their location in the oral cavity, the intestinal tract and the vagina (4). *Trichomonas vaginalis* is a flagellate parasite in the human vagina and urinary tract that causes mainly trichomonal vaginitis and urinary tract inflammation. The condition is an infectious disease caused mainly by sexual transmission (5). *Trichomonas hominis* is a flagellate parasite of the intestinal tract. *Trichomonas hominis* also has only a trophoblast stage and no cyst stage, and its shape is similar to that of *Trichomonas vaginalis*. Parasites in the human cecum and colon are more common in the ileocecum, so they are also commonly known as *Trichomonas hominis* or simply called *Pentarichomonas hominis* (6). At present, whether *Trichomonas hominis* is pathogenic to the human body is still inconclusive. It has been reported that the parasite can cause diarrhea, especially in infants and people with low immunity, and it may cause trichomonal enteritis. *Trichomonas tenax* is a flagellated protozoan (7) characterized by an inverted pear shape, averaging 6-10 μm in body length, with four anterior flagella and one posterior flagellum, along with a rhythmic undulating membrane. The parasite is commonly found in patients with inadequate oral hygiene and has been implicated in the progression of periodontal diseases (8). The current study presents a recent case in which *Trichomonas tenax* was detected in the pleural fluid of a patient with a lung tumor, offering insights for the future diagnosis and prevention of infectious diseases.

Case report

Patient. A 71-year-old man with a history of postoperative lung tumors presented with symptoms of chest tightness, shortness

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Key words: *Trichomonas tenax*, molecular identification, lung tumor, pleural effusion

of breath and discomfort when coughing upon admission to Heping Hospital Affiliated to Changzhi Medical College (Changzhi, China) in March 2024. Due to a malignant tumor of the right lung, the patient had undergone a thoracoscopic resection of the lower lobe of the right lung, a systematic lymph node dissection and a bullous ligation of the middle lobe of the right lung. The patient recovered well after the operation and was discharged from the hospital. A total of 16 days after the surgery, the patient suffered from chest tightness and shortness of breath. The expectorated sputum was white and sticky, which made it more difficult for the patient to cough. No obvious relief was experienced after resting. The patient's medical history revealed that the patient coughed uncomfortably when eating outside the hospital, and oral hygiene was poor. A laboratory test revealed an increase in white blood cells ($10.1x10^9$/l; normal range, 3.5-9.5x10^9/l; neutrophils, 91.8%; normal range, 40-75%) and a significant decrease in lymphocytes (0.52x10^9/l and 4.7%; normal range, 1.1-3.2x10^9/l and 20-50%) and hemoglobin (111 g/l; normal range, 130-175 g/l). A marked increase in BNP (1,728 ng/l; normal range, 0-125 ng/l) was also observed. The serum level of C-reactive protein (CRP) was increased (317.4 mg/l; normal range, 0-5 mg/l) and the level of procalcitonin (PCT) was slightly increased (0.16 ng/ml; normal range, 0-0.05 ng/ml). The results of the pleural effusion culture revealed *Gemella haemolysans* and anaerobes, including *Prevotella intermedia*, *Solobacterium moorei* and *Streptococcus oralis*. A chest radiograph and subsequent computed tomography (CT) revealed a pneumothorax in the right pleural cavity and a hydrothorax in the abdominal cavity, as shown in Fig. S1.

Routine examination of pleural effusion. The pleural effusion sample appeared yellow and turbid, and tested positive using the Fan Li method (9). A number of motile organisms resembling rotating worms were observed under a wet-film microscope, and numerous rotating worms were found via wet-film microscopy, as shown in Fig. 1. Some worms shook in place, with the flagella shaking quickly. Some swam directionally in the field of vision; some were slightly larger than white blood cells and flagella could be seen (Video S1).

Subsequently, the samples were centrifuged at x400 g for 5 min, stained with Wright-Giemsa for 10-15 min at 20-28˚C and examined under a microscope, as illustrated in Fig. 2.

On the basis of the morphological description, the organism was identified as *Trichomonas*.

PCR amplification and Sanger sequencing. To confirm that the pathogen was *Trichomonas tenax*, pleural effusion samples were sent to Wuhan University (Wuhan, China) for molecular detection. DNA purification from the samples was conducted via an Animal DNA Extraction kit (cat. no. BL1043A; Biosharp Life Sciences) following the manufacturer's protocol. For PCR detection of *Trichomonas*, the primers used were the PT3 forward primer (5'-AGTTCCATCGATGCCATTC-3') and the PT7 reverse primer (5'-GCATCTAAGGACTTAGAC G-3'), which target a 776-base pair region of the 18S ribosomal RNA, as previously described by Kikuta *et al* (1997) (10). For PCR amplification, 0.1 μg DNA template was mixed with 25 μl Super PCR Mix [2X Taq PCR MasterMix (with dyestuff); Beijing Solarbio Science & Technology Co., Ltd.] and 2 μl of each primer. The steps of PCR were as follows: Initial denaturation at 94˚C for 3 min, followed by 30 cycles of 94˚C for 30 sec, 55˚C for 30 sec and 72˚C for 1 min, with a final extension at 72˚C for 5 min. Subsequently, confirmation of the sequence as *Trichomonas tenax* was achieved via agarose gel electrophoresis. The amplicons were analyzed on 2% agarose gels (Fig. 3).

Finally, the samples were confirmed by Sanger sequencing, which identified the pathogen as *Trichomonas tenax*. A comparison of the nucleotide sequences from the samples with those in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) revealed 100% homology with *Trichomonas tenax*, and a comparison of the 18S rRNA gene sequences of *Trichomonas tenax* (accession number: D49495) was performed (Fig. 4).

The patient was administered metronidazole (at a dosage of 2 g per day, intravenously) four times a day for 7 days. The presence of trichomonads was negative from day 3 of metronidazole therapy. However, the patient's mixed infection status

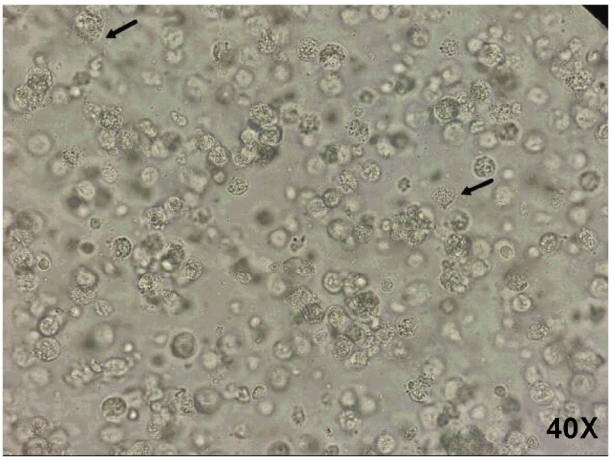

Figure 1. Wet-film microscopy. A number of rotating worms (arrows) were identified via wet-film microscopy.

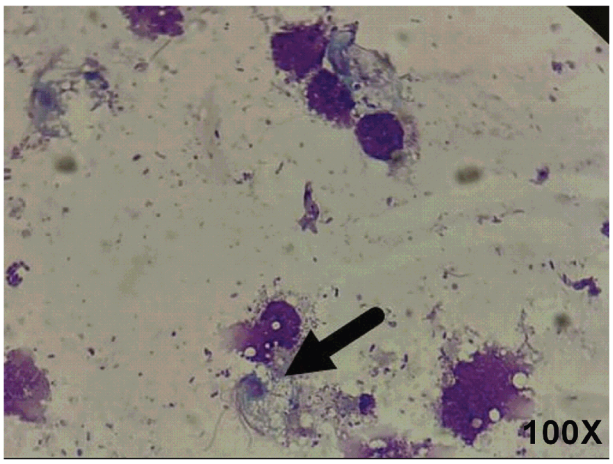

Figure 2. Giemsa staining. Samples were examined via light microscopy with Giemsa staining. The arrows indicate that a pear-shaped body with flagella was visible, containing a large dark purple long oval nucleus shaped like a mouse eye. There are four anterior flagella, and one posterior flagellum is as long as the undulatory membrane.

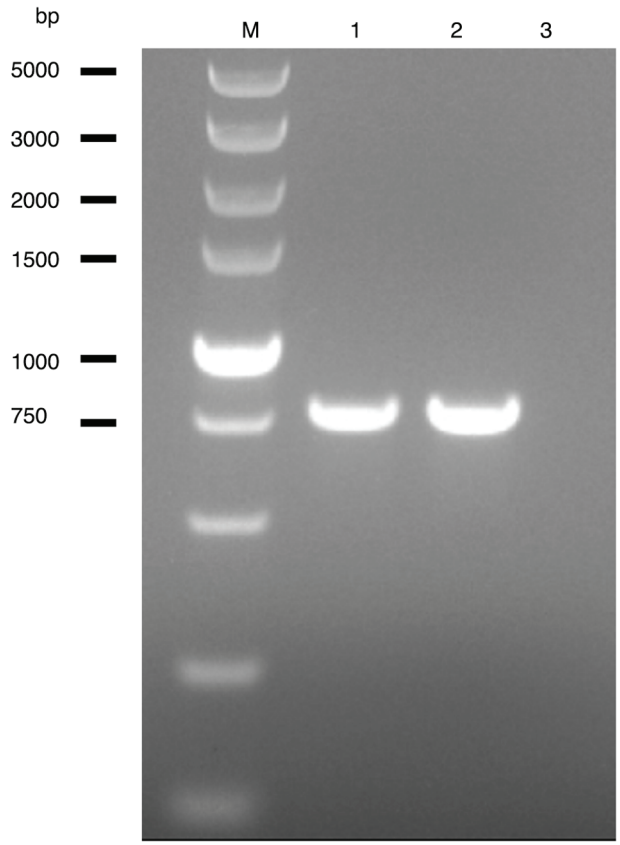

Figure 3. Gel electrophoresis of the PCR products. The PCR products were separated by gel electrophoresis of 2%. M, ladder (size marker), 100-5,000 bp; 1, PCR amplification products from the pleural effusion sample (concentration: 100 ng/μl; A260/280=1.78); 2, positive controls; 3, negative controls.

was serious, with bacterial infection in the early stage and fungal infection in the later stage, and the prognosis was poor.

Discussion

The average size of *Trichomonas tenax* is 7.1x4.7 μm. The parasite possesses four anterior flagella, which are often divided into two groups. The posterior flagellum is as long as the undulatory membrane. The undulatory membrane is shorter than the body, and the axial column is slender; most of the nuclei contain stained plasmids with a dark particle color (11). Parasites in the oral cavity, dental plaque and dental cavities, as well as trophozoites, spread through direct or indirect contact. Parasites often coexist with periodontal diseases such as gingivitis (12).

Trichomonas tenax parasitizes the oral cavity, dental plaque and cavities of the teeth. The parasites feed on bacteria, live in a slightly aerobic environment and are often accompanied by a large number of *Clostridium* and spirochetes. At present, the infection of lower respiratory tract caused by *Trichomonas tenax* is considered to be by inhalation via the oropharynx (13).

To the best of our knowledge, a report on oral diseases caused by oral *Trichomonas* infection exists (14), but few reports discuss infections affecting the lungs or other organs. According to the literature, *Trichomonas tenax* has been detected in the pleural effusion fluid in only 9 reported cases since 1966 (11,13,15-21). These patients included patients with various systemic diseases requiring immunosuppressive therapy, as well as patients with malignant tumors, as detailed in Table I. In the lungs, the proliferation of *Trichomonas tenax* appears to be facilitated by the presence of bacteria (13). As indicated in Table I, all 9 patients had concurrent infections of *Trichomonas tenax* with bacterial or fungal species, suggesting that *Trichomonas tenax* may utilize aerobic or anaerobic bacteria as a food source (11). Treatment with metronidazole was administered to all patients, resulting in mostly favorable outcomes; however, metronidazole is not without potential side effects (15-17) in humans.

The present study shares one case in which *Trichomonas tenax* was detected in a patient with pleural effusion. Pathogen metagenomics sequencing (PMseq) provides a direct and high-throughput method for sequencing infected samples, allowing comprehensive detection of microorganisms in clinical samples through detailed reports. Initially, PMseq (BGI Group) was conducted on pleural effusion samples, but *Trichomonas tenax* was not identified in the initial results. Some oral bacteria, including *Prevotella multiformis*, *Parvimonas micra*, *Fusobacterium nucleatum*, Porphyromonas endodontalis, *Dialister pneumosintes*, *Olsenella uli* and *Mogibacterium timidum*, were detected. At the same time, a large pneumothorax in the right pleural cavity was detected. Therefore, it is speculated that the *Trichomonas* in the patient's pleural effusion was likely inhaled into the lower respiratory tract from the oropharynx, which led to the aforementioned clinical symptoms. Clinically, there are also reports that most cases of pulmonary infection caused by *Trichomonas* are complicated with bacterial infection, which further supports the speculation that the *Trichomonas tenax* is most likely to come from the pharynx.

Upon consultation with technical support, it was determined that the sequencing database (Shenzhen Huada Medical Inspection Laboratory) did not include *Trichomonas tenax*. Subsequent reanalysis of the data revealed 306 reads corresponding to *Trichomonas tenax* in the non-human database. This underscores the importance of selecting an appropriate database, as the choice of database can significantly impact the accuracy of the final results.

The patients in the 9 literature cases were administered metronidazole (2 g intravenously per day) four times a day for 7 days. In some cases, metronidazole (1,000 mg) was administered orally twice a day for 5 days. In the present study, the presence of trichomonads was negative from day 3 of the institution's metronidazole therapy. However, this patient's mixed infection status was serious, with bacterial infection in the early stage and fungal infection in the later stage, and the prognosis was poor.

Quick and accurate identification is crucial for effective infection control. Trichomoniasis, which has long been neglected in scientific research, is now gaining renewed attention regarding its pathogenesis (22). Variations in the host-parasite interaction may involve human polymorphisms and environmental factors (23).

There is limited research available on *Trichomonas tenax*. In the present study, the parasite was identified via molecular methods. Among the diagnostic techniques available in laboratories, culture and molecular assays are considered the most

A

Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Select columns — Show 100

select all 100 sequences selected — GenBank | Graphics | Distance tree of results | MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Trichomonas tenax isolate Sputum11 18S ribosomal RNA gene, partial sequence	Trichomonas tenax	1330	1330	97%	0.0	99.06%	1461	JX943581.1
Trichomonas tenax isolate Sputum36 18S ribosomal RNA gene, partial sequence	Trichomonas tenax	1330	1330	97%	0.0	99.06%	1500	JX943576.1
Trichomonas tenax isolate Sputum10 18S ribosomal RNA gene, partial sequence	Trichomonas tenax	1330	1330	97%	0.0	99.06%	1542	JX943572.1
Trichomonas tenax 16S-like ribosomal RNA, complete sequence	Trichomonas tenax	1330	1330	97%	0.0	99.06%	1580	U37711.1
Trichomonas canistomae 16S small subunit ribosomal RNA gene, partial sequence	Trichomonas ca…	1327	1327	97%	0.0	98.93%	1507	AY247748.1
Trichomonas tenax isolate Sputum13 18S ribosomal RNA gene, partial sequence	Trichomonas tenax	1319	1319	97%	0.0	98.79%	1530	JX943577.1

B

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Query  35   GGTACAGTTTTCCACTGTACCGAAACCTAGCAGAGGGCCAGTCTAGTGCCAGCAGCTGCG  94
            |||||  |||||  |  |||||  ||  ||||||||||||||||||||  ||||||||||||||||
Sbjct  8    GGTAC-GTTTT-C-CTGTA-CG-AACCTAGCAGAGGGCCAGTCTGGTGCCAGCAGCTGCG  62

Query  95   GTAATTCCAGCTCTGCGAGTTTGCTCCCATATTGTTGCAGTTAAAACGCCCGTAGTCTGA  154
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  63   GTAATTCCAGCTCTGCGAGTTTGCTCCCATATTGTTGCAGTTAAAACGCCCGTAGTCTGA  122

Query  155  ATTGGCCAGCAATGGTCGTATGTATTTATACGTTCACTGTGAACAAATCAGGACGCTTAG  214
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  123  ATTGGCCAGCAATGGTCGTATGTATTTATACGTTCACTGTGAACAAATCAGGACGCTTAG  182

Query  215  AGTATGGCTACATGAATGACTCAGCGCAGTATGAAGTCTTTGTTTTCTTCCGAAAACAAG  274
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  183  AGTATGGCTACATGAATGACTCAGCGCAGTATGAAGTCTTTGTTTTCTTCCGAAAACAAG  242

Query  275  CTCAATGAGAGCCATCGGGGGTAGATCTATCTCATGACGAGTGGTGGAATACTTTGACTC  334
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  243  CTCAATGAGAGCCATCGGGGGTAGATCTATCTCATGACGAGTGGTGGAATACTTTGACTC  302

Query  335  ATGAGAGAGAATCTGAGGCGAAGGCGTCTACCTAGAGGGTTTCTGTCGATCAAGGGCGAG  394
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  303  ATGAGAGAGAATCTGAGGCGAAGGCGTCTACCTAGAGGGTTTCTGTCGATCAAGGGCGAG  362

Query  395  AGTAGGAGTATCCAACAGGATTAGAGACCCTGGTAGTTCCTACCTTAAACGATGCCGACA  454
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  363  AGTAGGAGTATCCAACAGGATTAGAGACCCTGGTAGTTCCTACCTTAAACGATGCCGACA  422

Query  455  GGAGTTTGTCACTTGTTAGTGGCAGAATCTTTGGAGAAATCATAGTTCTTGGGCTCTGGG  514
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  423  GGAGTTTGTCACTTGTTAGTGGCAGAATCTTTGGAGAAATCATAGTTCTTGGGCTCTGGG  482

Query  515  GGAACTACGACCGCAAGGCTGAAACTTGAAGGAATTGACGGAAGGGCACATCAGGGGTGG  574
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  483  GGAACTACGACCGCAAGGCTGAAACTTGAAGGAATTGACGGAAGGGCACACCAGGGGTGG  542

Query  575  AGCCTGTGGCTTAATTTGAATCAACACGGGGAAACTTACCAGGACCAGATGTTTTTTATG  634
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  543  AGCCTGTGGCTTAATTTGAATCAACACGGGGAAACTTACCAGGACCAGATGTTTTTTATG  602

Query  635  ACTGACAGGCCTTGGGTCCTTCAGGATAATTCTTTTGGTGGTGCATGGCCGTTGGTGGTG  694
            ||||||||||  |||||||  |||||||||  |  ||||||||||||||||||||||||||||||||
Sbjct  603  ACTGACAGGCTTTGGGTCTTTCAGGATATTGCTTTTGGTGGTGCATGGCCGTTGGTGGTG  662

Query  695  CGTGGGTTGACCTGTCTAGCGTTGATTCAGCTAACGAGCGAGATTATCGCCAATTATTTA  754
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  663  CGTGGGTTGACCTGTCTAGCGTTGATTCAGCTAACGAGCGAGATTATCGCCAATTATTTA  722

Query  755  CTTCGTCTAAGTCCTT-AGATGC  776
            ||||||||||  |||||  ||||||
Sbjct  723  CTTCGTCTAA-TCCTTTAGATGC  744
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Figure 4. Comparisons of the nucleotide sequences of the samples with the GenBank database. (A) Comparisons of the nucleotide sequences of the study samples with those in the GenBank database. (B) 'Query' represents 18S rRNA gene sequences of *Trichomonas tenax* (Gene Bank accession number: D49495). 'Sbjct' represents the PCR amplification products from the pleural effusion sample.

Table I. Clinical and microbiological characteristics of the 9 cases of *Trichomonas tenax* detected in pleural effusion published in the literature since 1968.

First author, year	Age, years	Sex	Underlying disease(s)	Immunosuppressive therapy	Coinfection pathogen	Treatment	Outcome	(Refs.)
Memik, 1968	87	M	Chronic pulmonary disease	No	Bacteria	MTZ and TET	Clinical improvement	(15)
Ohkura *et al*, 1985	70	M	Alcohol abuse	No	Bacteria	MTZ, CEF	Clinical improvement	(16)
Shiota *et al*, 1998	53	M	Acromegaly rectal adenocarcinoma	Chemotherapy, corticotherapy and cobalt irradiation	Bacteria	MTZ	Clinical improvement	(17)
Porcheret *et al*, 2002	59	M	Lung adenocarcinoma	Corticotherapy	Bacteria	MTZ, GEN and CIP	Death	(18)
Mallat *et al*, 2004	58	M	Esophagus adenocarcinoma	No	Bacteria	MTZ, PTZ and GEN	Death	(19)
Bellanger *et al*, 2008	33	F	Heart transplantation	Yes	Bacteria	MTZ and PTZ	Clinical improvement	(20)
Leterrier *et al*, 2012	67	F	Glioblastoma high	Corticotherapy	Bacteria	MTZ	Death	(13)
Wu *et al*, 2021	16	M	Cerebral palsy	No	Fungus	MTZ and VOR	Clinical improvement	(11)
Cai and Fang, 2022	69	M	Cerebral infarction	No	Bacteria	MTZ and PTZ	Clinical improvement	(21)

M, male; F, female; MTZ, metronidazole; TET, tetracycline; CEF, cephalotin; GEN, gentamicin; CIP, ciprofloxacin; PTZ, piperacillin-tazobactam; VOR, voriconazole.

effective for detecting parasites (24). Culture is typically the gold standard for identifying pathogenic microbes in infections (25), despite its drawbacks, such as low sensitivity and specificity, and time-consuming processes. By contrast, PCR has greatly advanced microbial identification due to its high sensitivity and specificity. PCR allows for quick determination of a microbial presence in small samples (12). It is recommended that future studies employ molecular methods, as relying solely on microscopy can lead to underestimation of the true prevalence of oral parasites. Further research is needed to explore the molecular epidemiology of these oral parasites.

Oral *Trichomonas* species predominantly inhabit anaerobic environments, such as gingival crevicular spaces (26). When the host's immunity decreases, it creates a environment that is conducive to the proliferation of pathogenic bacteria, facilitating the growth and reproduction of *Trichomonas tenax*. The protozoa metabolize host epithelial cell glycogen through glycolysis to obtain energy, further promoting the growth of pathogenic bacteria. Consequently, patients, especially those undergoing chemotherapy or radiotherapy for tumors, may experience concurrent oral *Trichomonas* infections alongside various pathogenic bacteria. Chemotherapy and radiotherapy can induce side effects such as neutropenia and compromised humoral and cellular immunity, which increase susceptibility to severe bacterial, viral and parasitic infections (12,27).

The potential pathways for *Trichomonas tenax* infection can be defined on the basis of published literature on pulmonary *Trichomonas tenax* (13,28) as follows: i) Primary or secondary immunocompromised conditions (29), including but not limited to solid tumors, hematological malignancies, rheumatic diseases, use of immunosuppressive agents, long-term systemic corticosteroid use, and transplantation of solid organs and hematopoietic stem cells. ii) Oral/periodontal diseases, risk factors for aspiration and any procedure of a therapeutic or diagnostic nature related to the upper thorax or esophagus. The third risk factor is with regard to *Trichomonas tenax* infection in the lungs, given the residence of *Trichomonas tenax* in the human oral cavity.

The results of the study by Kooshki *et al* revealed a high frequency of oral cavity parasites in children who were diagnosed with malignancies or who were receiving treatment with chemotherapy in Lorestan Province, Iran (3). Renal disorders increase the susceptibility of patients to infections, including those caused by oral cavity infections (28).

The present case involved a postoperative patient with lung cancer, and in association with the immune status of the patient, the patient had poor immunity, which provides an opportunity for pathogens, including oral *Trichomonas* and other potential co-infectious pathogens (such as bacteria, fungi and viruses), to escape immunity. In addition, it is worth considering whether lung cancer surgery provides opportunities for oral *Trichomonas* to enter the lower respiratory tract, and whether there is the possibility of open communication between the digestive system and chest cavity of patients with lung cancer. Finally, chest CT on admission revealed a large pneumothorax in the right pleural cavity, which provided an opportunity for the oral *Trichomonas* to enter the lungs.

As a result, oncologists and dental practitioners need to be vigilant in managing oral health issues in patients susceptible to various infections, with

particular emphasis on those undergoing cancer treatment. Laboratory professionals should maintain vigilance and careful observation to avoid overlooking positive indicators of infections.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

JW, QZ, YN and AJ conceptualized the study. JW, QZ, LD and YY performed the investigation. JW, QZ and DL were responsible for the wet-film microscopy, Giemsa staining and gel electrophoresis of the PCR products. JW performed the formal analysis. JW wrote the initial draft. JW, QZ, YN, LD, JZ and AJ reviewed and edited the manuscript. All authors have read and approved the manuscript. JW, QZ, DL and AJ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

The patient provided written informed consent for publication.

Competing interests

The authors declare that they have no competing interests.

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