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Editor's Pick | Parasitology | Minireview

Lophomonas as a respiratory pathogen—jumping the gun

Abhishek Mewara,[1] Gillian H. Gile,[2] Blaine Mathison,[3,4] Huan Zhao,[5] Bobbi Pritt,[6] Richard S. Bradbury[5]

AUTHOR AFFILIATIONS See affiliation list on p. 14.

ABSTRACT Human infections with the protozoan *Lophomonas* have been increasingly reported in the medical literature over the past three decades. Initial reports were based on microscopic identification of the purported pathogen in respiratory specimens. Later, a polymerase chain reaction (PCR) was developed to detect *Lophomonas blattarum*, following which there has been a significant increase in reports. In this minireview, we thoroughly examine the published reports of *Lophomonas* infection to evaluate its potential role as a human pathogen. We examined the published images and videos of purported *Lophomonas,* compared its morphology and motility characteristics with host bronchial ciliated epithelial cells and true *L. blattarum* derived from cockroaches, analyzed the published PCR that is being used for its diagnosis, and reviewed the clinical data of patients reported in the English and Chinese literature. From our analysis, we conclude that the images and videos from human specimens do not represent true *Lophomonas* and are predominantly misidentified ciliated epithelial cells. Additionally, we note that there is insufficient clinical evidence to attribute the cases to *Lophomonas* infection, as the clinical manifestations are non-specific, possibly caused by other infections and comorbidities, and there is no associated tissue pathology attributable to *Lophomonas*. Finally, our analysis reveals that the published PCR is not specific to *Lophomonas* and can amplify DNA from commensal trichomonads. Based on this thorough review, we emphasize the need for rigorous scientific scrutiny before a microorganism is acknowledged as a novel human pathogen and discuss the potential harms of misdiagnoses for patient care and scientific literature.

KEYWORDS parasites, misidentification, *Lophomonas*, flagellate, cilia, ciliocytophthoria

Over the past three decades, there has been a noticeable increase in the medical literature of reports of human infections with *Lophomonas* species, a parabasalid flagellate protozoan that resides in the intestines of cockroaches. Following the first report of *Lophomonas*-caused human infection in 1993 (1), a number of additional cases were reported from China. These reports were based on microscopy of respiratory specimens wherein unidentified cells were presumptively identified as *Lophomonas* based solely on their shape, size, extracellular organelles (cilia/flagella), and motility. These reports primarily described pneumonia- and respiratory system illness-like symptoms, but also sinus and urinary tract infections (2–5). Later, it became apparent to many workers that ciliated bronchoepithelial cells were being misidentified as *Lophomonas* (6–8). However, *Lophomonas* continues to be reported sporadically as a cause of human disease from around the globe.

In 2019, a polymerase chain reaction (PCR) was developed to detect the *18S rRNA* sequence of *Lophomonas blattarum* for the first time in Iran (9); as a result, many more cases of *Lophomonas* in respiratory samples were reported from that country. However, the published sequences from these reports are not highly similar (79.56–86.21%) to the published sequences of known *Lophomonas* from cockroaches. They are more closely related to *Tetratrichomonas* and *Pentatrichomonas* (Trichomonadida and Parabasalia), which are distant relatives of *Lophomonas* (10, 11). Moreover, after the development of

Editor Romney M. Humphries, Vanderbilt University Medical Center, Nashville, Tennessee, USA

Address correspondence to Abhishek Mewara, abhishekmewara@gmail.com, or Bobbi Pritt, Pritt.bobbi@mayo.edu.

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this PCR, subsequent reports began omitting microscopy images and publishing only PCR positivity data, making it even more challenging to confirm the authenticity of these diagnoses. After excluding Chinese language articles, a recent systematic review of studies described 307 lophomoniasis cases between 1993 and 2020 from 10 countries on four continents (12). Iran has the most reported cases, followed by China, Panama, Turkey, India, Mexico, Spain, Egypt, Malaysia, and Peru (12).

In this review, we examine the various aspects of the putative role of *Lophomonas* in causing human disease, including the morphology of *Lophomonas* species in relation to bronchial ciliated epithelial cells, heterogeneity in the clinical reports, pitfalls in their molecular identification by PCR, lack of evidence of its pathogenicity in human tissues or animal models, and the potential harm of such misdiagnoses in patient care and scientific literature.

THE PARABASALID *LOPHOMONAS*

Lophomonas are multiflagellated protozoa in the phylum Parabasalia. They inhabit the hindguts of common pest cockroaches, including *Periplaneta americana* (American cockroach), *Blatta orientalis* (Oriental cockroach), and *Blattella germanica* (German cockroach) (13, 14). Little is known about their biology, except that they live under anoxic conditions in the cockroach gut, they can form cysts, and they appear to have a commensal, not parasitic, relationship with their hosts (13, 15). Trichonymphida (Parabasalia), which are obligate symbionts of termites, are their closest relatives (10). The genus *Lophomonas* contains two valid species, *L. blattarum* Stein and *L. striata* Bütschli (16, 17). A third species, *L. sulcata* Schuster, was described, but it is considered to be a synonym of *L. striata* (15, 18). There are no cultures available for either of these species, although temporary cultures of *L. blattarum* have been achieved on horse serum slants with a liquid overlay of ATCC 802 medium (11, 19).

The morphological features of *L. blattarum* and *L. striata* were first described in the 19th century and presented with only line drawings (16, 17). More detailed descriptions were published in the 1920s, again with only light microscopy and line drawings (15), and then in the 1960s with transmission electron microscopy (EM) (19). Phase contrast light microscopy and scanning electron microscopy of *L. blattarum* were presented more recently (20). The main distinguishing morphological characteristic of *Lophomonas* is the apical tuft of numerous flagella (~50 or more). These emerge from a small, incomplete circle of basal bodies at the extreme cell apex and are tightly bundled together, reminiscent of a horse's tail. They are never distributed across a surface like a brush or a lawn. The nucleus is located directly beneath the tuft of flagella and is enclosed by a microtubular structure known as a calyx or chalice (15). These characteristics can be observed in living, unstained specimens with contrast-enhancing optics such as phase or differential interference contrast (Fig. 1A through C). At the base of the calyx, the microtubular axostyle extends toward the cell posterior, like the stem of a wine glass. Scanning and transmission EM have revealed the fine structure of the calyx and its tightly ordered relationship with endomembrane vesicles (20). However, EM studies have rarely been utilized to differentiate *Lophomonas* from bronchial epithelial cells in clinical samples (7).

Despite sharing these characteristics, *L. striata* and *L. blattarum* are morphologically quite distinct. *L. blattarum* cells are spherical to oval or occasionally pyriform and measure 15–30 µm in length, whereas *L. striata* cells are elongate and spindle-shaped and measure 30–60 µm in length, due to their thick coating of fusiform ectosymbiotic bacteria (15). So far, only *L. blattarum* has been reported from human samples, perhaps unsurprisingly since *L. striata* cells are more distinctive and therefore less likely to be mistaken for human epithelial cells.

HUMAN CILIATED EPITHELIAL CELLS

Ciliated epithelium is found in various parts of the human body, including the respiratory tract (parts of nasal cavity and trachea down to the terminal bronchioles of the lung)

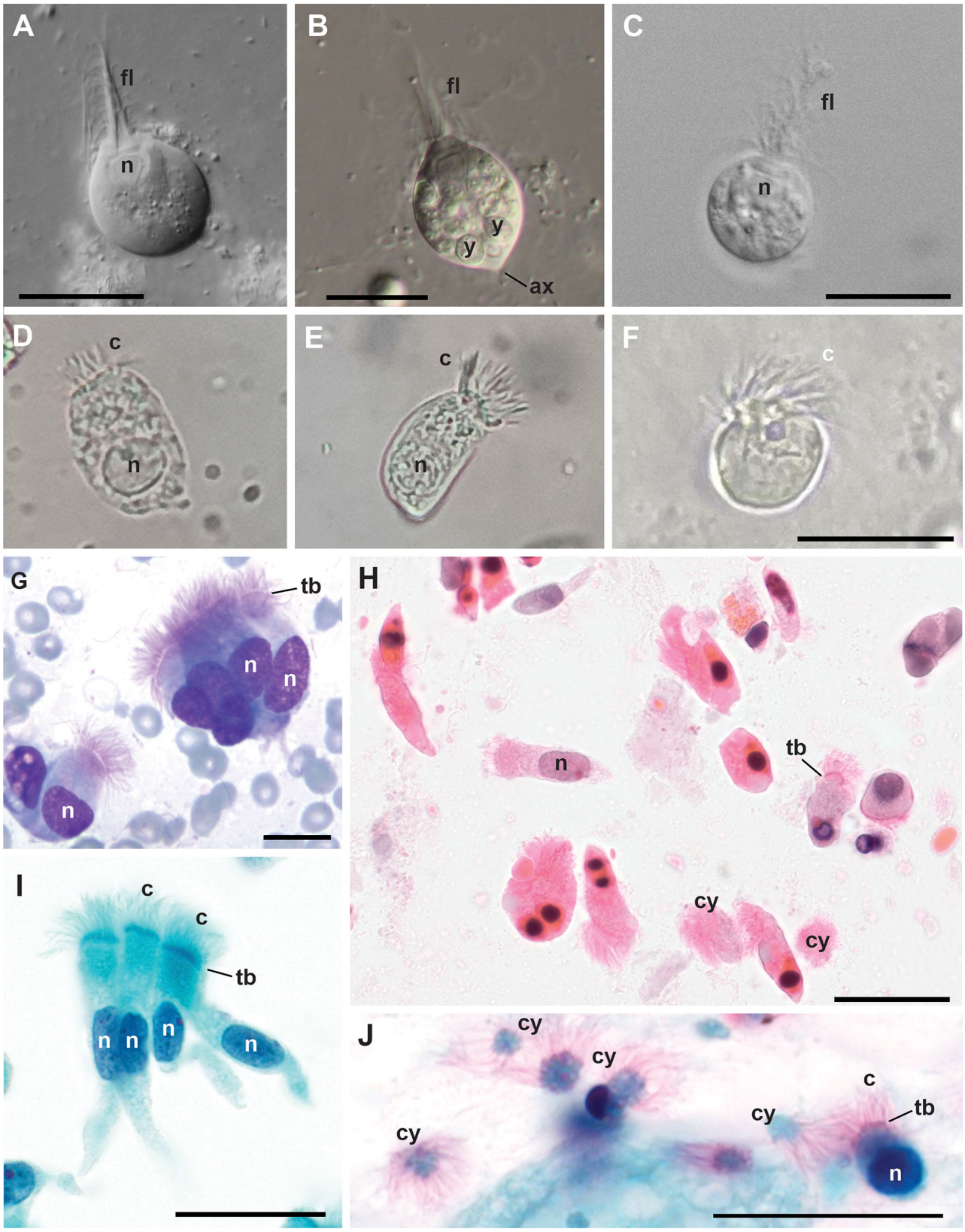

FIG 1 Light microscopic comparison of *Lophomonas blattarum* to human ciliated epithelial cells. (A–C) *L. blattarum* cells, unstained wet mounts, differential interference contrast microscopy. Note the concentrated tuft of long flagella subtended by the anteriorly located nucleus. A slight posterior protuberance formed by the distal tip of the axostyle is visible in (B). (A) Cell from a temporary culture with rice starch added to the medium. (B) Cell from a temporary culture

(Continued on next page)

FIG 1 (Continued)

with yeast added to the medium. Several ingested yeast cells are visible in the cytoplasm. (C) Cell isolated directly from a *Blatta orientalis* gut. (D–F). Human ciliated epithelial cells in respiratory specimens, unstained saline wet mounts, phase contrast microscopy. Note the distributed tuft of equal length, short cilia, covering the apical surface of the cell. A large, posteriorly located nucleus is visible in (D) and (E). (G–J) Fixed, stained mounts of intact, and degenerated ciliated epithelial cells. (G) Diff-Quik stained, air-dried preparation of normal ciliated respiratory epithelial cells mechanically exfoliated by bronchial brushing. Cell nuclei measure approximately 12–14 µm in diameter. (H) Papanicolaou-stained sputum specimen preserved in CytoLyt medium demonstrating numerous detached ciliated respiratory epithelial cells in various states of degeneration. Note that the terminal bar is readily apparent in many of the cells, allowing them to be readily recognizable as ciliated human cells. (I) Papanicolaou-stained bronchial brushing specimen preserved in CytoLyt medium demonstrating mechanically detached, normal, ciliated respiratory epithelial cells. Note the columnar shape with narrowed basilar region where the cell was previously attached to the basement membrane. (J) Papanicolaou-stained endocervical specimen preserved in CytoLyt medium demonstrating abundant detached ciliary tufts (ciliocytophthoria), in which only the cilia and terminal bar are apparent. Abbreviations: flagella (fl), nucleus (N), yeast cell (Y), protuberance due to axostyle (ax), cilia (C), terminal bar (tb), ciliocytophthoria (cy). All scale bars = 20 µm.

(Fig. 1D through J), male and female genital tract (ductus epididymitis and oviduct), and central nervous system (ventricles) (21). Ciliated cell metaplasia (appearance of a tissue type normally found elsewhere in the body) can also occur in different epithelia throughout the body.

Various disease states, such as infection, inflammation, and malignancy, can result in the exfoliation of individual cells or clusters of cells (termed creola bodies in respiratory secretions), which can then be detected in clinical specimens such as sputum. Occasionally, the cilia-bearing apical portion of the cell is separated from the nucleated cytoplasm resulting in the presence of detached ciliary tufts (DCTs) in respiratory specimens. This phenomenon was termed ciliocytophthoria in 1956 by Dr. Papanicolaou, who noted its association with respiratory viral infections and pulmonary malignancy (22, 23).

In microscopic examination, degenerating ciliated epithelial cells, creola bodies, and DCTs may be misdiagnosed as *Lophomonas*, particularly when motile cells are viewed in unfixed wet mount preparations. These cells may be present in any sample containing ciliated cells and have been reported from urine, semen, peritoneal washings, cerebrospinal fluid, and an epididymal cyst aspirate (24–26).

DATABASE AND SEARCH STRATEGY

Screening of studies

For the systematic analysis of *Lophomonas* literature, we searched Medline and Embase for English, and CNKI for Chinese language articles up to 16 May 2023 (Search query: *Lophomonas*). The publication details were entered into an Excel spreadsheet, followed by screening the title, abstract, and full text. After removing duplicates and conference abstracts, a total of 100 articles including 55 clinical case reports and case series, 21 original research studies, 17 letters to the editor and commentaries, and seven reviews were accessed (Table S1; Fig. 2). Then, the studies were screened for any type of images or videos (microscopy, radio imaging, and gel electrophoresis), and clinical data (either from the full text or abstract if the full text was unavailable or in a language other than English or Chinese). A total of 76 studies with images and/or video and 47 studies with clinical data were screened based on the following inclusion and exclusion criteria (Fig. 3).

Criteria of studies for image and video analysis

Studies with images and/or videos of putative *Lophomonas* from human or cockroach samples and ciliated epithelium were included.

Criteria of studies for clinical data analysis

Studies reporting human cases with clinical data, with *Lophomonas* infection diagnosed by microscopy (with images) or PCR were included. Studies that only summarized data from other cases, reported the same case(s), and lacked a microscopy image or

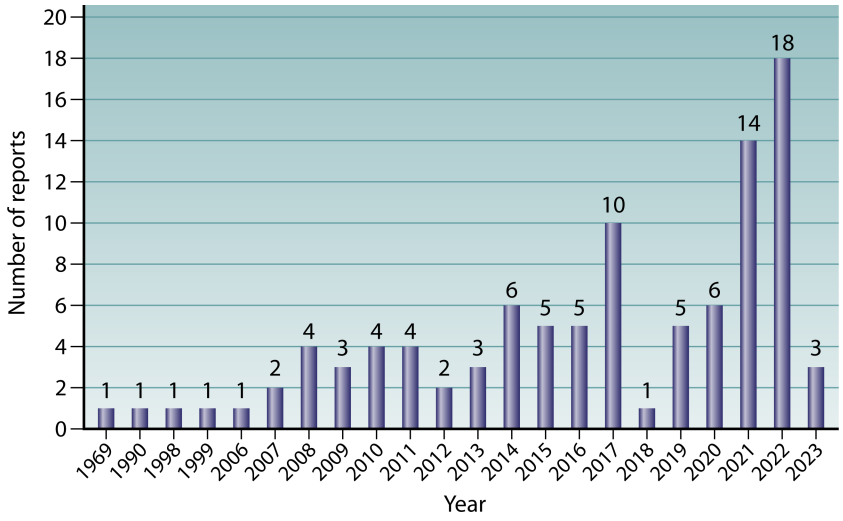

FIG 2 Reports of *Lophomonas* on Medline, Embase, and CNKI databases (till 16 May 2023).

PCR analysis were excluded. For case reports that also include summaries of previous studies, only the clinical data of the individual case was included, while the summaries of other cases were excluded. The retrospective or case-control studies with relatively large number of patients were selected for the meta-analysis to estimate the pooled prevalence of putative *Lophomonas* infections.

Data extraction

The following information was extracted: authors, country, patient demographics, clinical features, radiology features, diagnostic method, treatment, and outcome. The images and videos were acquired from the publications and reviewed by all the authors.

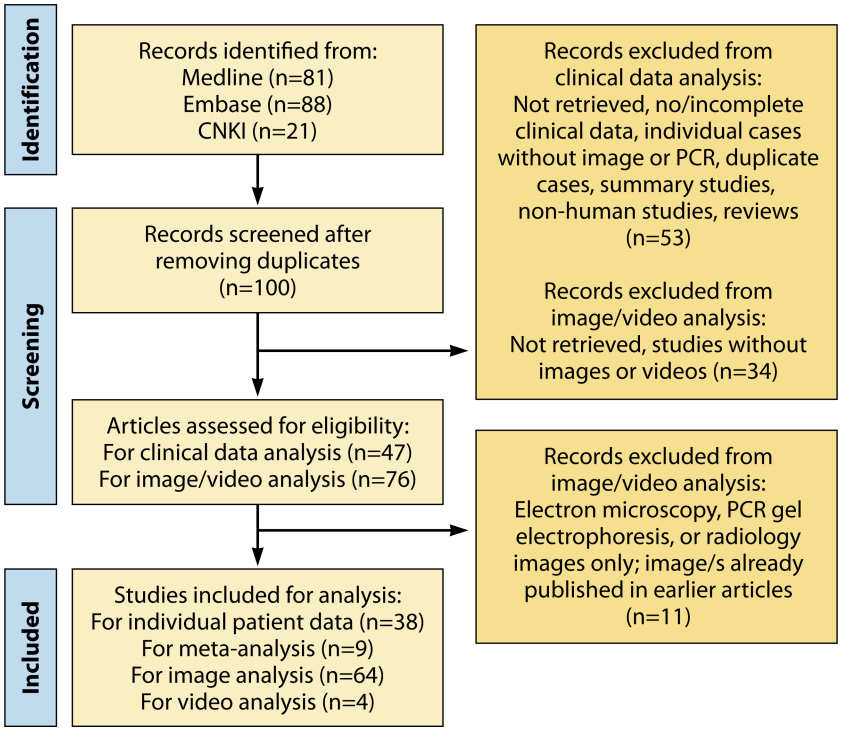

FIG 3 PRISMA flowchart for inclusion of studies on *Lophomonas*.

IMAGE AND VIDEO ANALYSIS

Out of 76 studies with images and/or videos of putative *Lophomonas*, a total of 65 studies were selected for the image (n = 64) and video analysis (n = 4) (Fig. 2).

Microscopy image analysis

Of the 64 studies included for the image analysis, 49 contained images of human samples, 10 contained images that did not directly report any human case, and 5 contained images of cockroach specimens (Table S2). The microscopy-based images of the studies were sorted for the analysis giving 79 image sets that were reviewed by all the authors. The image sets were classified based on the identifying features of the image as "*Lophomonas*," "Not *Lophomonas*," and "insufficient diagnostic features/low quality images."

Of the 79 sets of images, 42 did not show features of *Lophomonas*, 34 were of low quality or with insufficient diagnostic features, and only 3 image sets resembled true *Lophomonas* species; these were from cockroach specimens. Thus, none of the images from the human samples demonstrated definitive *Lophomonas* characteristics such as a tight anterior bundle of flagella, an anterior nucleus, the calyx surrounding the nucleus, or a posteriorly protruding axostyle. A majority of the *Lophomonas* image sets from human samples had features that clearly rule out that identification, such as short, even length cilia emerging from across a broad, flattened apical surface, a columnar cell shape, a large posteriorly located nucleus, and/or a "terminal bar" visible along the cell's anterior surface. Thus, the images included features demonstrating ciliocytophthoria or otherwise detached epithelial cells from the human respiratory tract. Several reports neither displayed definitive *Lophomonas* characteristics nor characteristics that would rule out *Lophomonas*. In three of the studies, the authors used light microscopy images and other techniques such as metagenomics (27) and electron microscopy (7, 28) to rule out the presence of *Lophomonas* in respiratory samples.

Of the six image sets from cockroach specimens (from five studies), three sets resembled true *Lophomonas*, while three sets were of low quality or with insufficient diagnostic features. Of the three studies convincingly demonstrating true *Lophomonas*, two provided light micrographs of *L. striata*, one from Canada, and one from China (misidentified in the report as *L. blattarum*). In both studies, the *L. striata* images feature an apical bundle of flagella, and the spindle-shaped cells are covered with ectosymbionts, giving them a longitudinally striated appearance (10, 29). As mentioned previously, *L. striata* is quite distinctive in appearance and therefore unlikely to be confused with ciliated epithelial cells. The third study from Spain features a cell of the expected size and shape with a pointed posterior and a clear flagellar bundle that emerges from the cell apex, consistent with the morphology of *L. blattarum* (30). This report also includes images of cysts attributed to *L. blattarum*. While *L. blattarum* is expected to encyst, no other micrographs of *L. blattarum* cysts were found in our literature search, so it is unclear how the diagnosis of these cysts was made.

Video analysis

For video analysis, 15 videos were analyzed from four studies (Table S3). In all of these videos, the relatively short cilia emerge in parallel from a relatively broad surface; they do not form a bundle arising from a concentrated point as in *Lophomonas*. These cells are rocking in place with coordinated, symmetrical ciliary beats, and cannot be seen swimming as a true *Lophomonas* cell would (Video S1 and S2). In a free medium, the human ciliated epithelial cells can be observed to rock in place and/or propel themselves slightly backward (away from the cilia) due to the vigorous ciliary waving and lack of a basement membrane to hold them, while a true *Lophomonas* would actively swim in the direction of the flagella. Thus, all these features exclude these cells from being *Lophomonas*. In one study, two videos demonstrated that ciliated epithelial cells can continue to move long after detaching from the airway epithelium (8)

For comparison, a video of the movement of the true *L. blattarum* from a cockroach is provided (Video S3). It can be observed that the flagella of *Lophomonas* arise from a concentrated point, rather than being spread out over a surface, and form a conspicuous bundle. The cell swims in the direction of its flagella using a coordinated flagellar beat that includes a distinct effective stroke and recovery stroke. The axostyle can be seen protruding from the cell posterior. At certain moments, the calyx can also be seen as it comes into the plane of focus.

Comments/conclusions on image/video analysis

In the literature, there are numerous low-quality images of the putative "*Lophomonas*." While the majority of the published images of human samples can be clearly identified as human ciliated epithelial cells, the others either lack any morphological feature consistent with *Lophomonas*, or are of low quality and lack any useful diagnostic features. Depending on how the clinical specimens were collected and issues with fixation and processing, there may be morphological alterations such as a rounding up of some of the epithelial cells or a distention of the basal portion which might be mistaken for the axostyle of *Lophomonas*. During real-time microscopic analysis of a respiratory specimen, the motility of the cells is an important diagnostic feature that is lost when still images are captured. Since the ciliated epithelium may continue to show movement for a long period after detachment from host airway epithelium and thus may be confused with a live cell (8), the differentiation of the type of motility is important. Such differentiation is also especially useful in the absence of microscopes with contrast-enhancing optics, because the swimming characteristics are readily visible even without these. When observing non-motile cells in a sample, a live cell stain like DAPI (4′,6-diamidino-2-phenylindole) that stains the nucleus may be used. If there is no nucleus at the apex of the cell, directly beneath the bundle of flagella, *Lophomonas* can be ruled out. In the future, it is recommended that the studies reporting *Lophomonas* from human specimens should include both videos and multiple still micrographs to provide convincing evidence of the identification. The still images must be of high quality and must display the essential diagnostic features as enumerated above.

CLINICAL ANALYSIS

Out of 47 studies with clinical data, 38 studies reporting 58 human cases presumed to have been diagnosed with *Lophomonas* infection by microscopy (with images) and/or molecular tests were analyzed for clinical data of individual cases. The qualitative data were summarized using percentages and frequency, while the quantitative data were summarized using mean, median, and standard deviations. In order to estimate the pooled prevalence of putative *Lophomonas* infections with 95% confidence interval (CI), a meta-analysis was performed on nine retrospective or case-control studies with larger number of cases (total 346) using RStudio software (version 3.4). Heterogeneity among the effect sizes of the studies was evaluated by *I*-square (I^2) test; wherein an observation of I^2 more than 75% is considered as high heterogeneity. A Doi plot was used to evaluate the publication bias. The overall year- and country-wise distribution of patients diagnosed with *Lophomonas* infection included in the clinical analysis (n = 58) and meta-analysis (n = 346) in this study are shown in Table 1.

Clinical data analysis

Fifty-eight cases that were included in the individual case analysis were reported from eight countries (Table S4). The median age of patients was 38.65 (± 22.85) years, ranging from 3 months to 79 years, and there were 31 females and 27 males. The most common presenting symptoms were cough (41, 70.69%), followed by fever (35, 60.35%), expectoration (27, 46.55%), breathlessness (24, 41.38%), chest pain (17, 29.31%), and others (Table 2). Of these, 48 (82.76%) were diagnosed by microscopy of BAL samples, 6 (10.35%) on sputum samples, and 6 also showed *Lophomonas* in other samples; while 20

TABLE 1 Year- and country-wise distribution of patients diagnosed with *Lophomonas* infection included in the clinical analysis (*n* = 58) and meta-analysis (*n* = 346) for this study

Year	Country								
	Iran	China	India	Ecuador	Peru	Turkey	Malaysia	USA	Total
2006		4							4
2008		2							2
2010		1							1
2011		3							3
2012		1							1
2014		3							3
2015			1						1
2016	1		1						2
2017		119	1						120
2019	1					1	1		3
2020	4	1	12						17
2021	47	2		6	1				56
2022	186	1			1				188
2023	1		1					1	3
Total	240	137	16	6	2	1	1	1	404

(34.48%) were diagnosed by PCR. Among the samples other than BAL and sputum, there were two tissue samples, including a transbronchial biopsy and a surgical piece—images from both samples did not demonstrate any tissue pathology features.

Comorbidities or associated conditions were present in 52 (89.66%) patients. Among these were acute myelocytic leukemia, lung adenocarcinoma, allergic rhinitis, asthma, breast cancer, bronchitis, bronchomalacia, chronic obstructive pulmonary disease, chronic renal failure, common variable immunodeficiency, diabetes mellitus, hypertension, lung fibrosis, pneumonia, lung carcinoma, renal cell carcinoma, renal transplant, tuberculosis, scleroderma, systemic lupus erythematous, as well as bacterial, viral and parasitic infections such as aspergilloma, *Candida* species, SARS-CoV-2, cryptococcosis, cytomegalovirus, echinococcosis, *Klebsiella* species, *Pneumocystis jirovecii*, *Pseudomonas aeruginosa*, and tuberculosis (Table S1).

Of the 58 patients, 54 (93.10%) received metronidazole for the treatment of the putative *Lophomonas*, 2 received ornidazole and 1 tinidazole, while 33 (56.89%) also received other antimicrobial agents concurrently for their illness. A total of 35 (60.35%) patients showed improvement after starting metronidazole, while 3 (5.17%) showed deterioration or relapse, and 6 (10.35%) died (Table 2).

The meta-analysis of nine studies included a total of 346 putative *Lophomonas* infections among 2,743 individuals with an estimated pooled prevalence of 17% (95% CI: 8–29%) as shown in the forest plot in Fig. 4; a summary of these studies is provided in Table S5. Seven of these studies were from Iran, and one each from China and India. In the meta-analysis, a substantial heterogeneity among the studies was observed (I^2 = 98%, P < 0.01), which indicates that the results of the studies are diverse and do not align with each other. The Doi plot shows a symmetrical distribution of the studies included in the meta-analysis (Fig. S1A). The regression line in the Bubble plot given in Fig. S1B shows a negative association between the sample size and the prevalence of *Lophomonas* cases for the included studies.

Comments/conclusions on clinical analysis

The clinical data of *Lophomonas* reports are substantially heterogeneous. The clinical manifestations are non-specific, indicate the involvement of both the upper and lower respiratory systems and are likely to be present in respiratory illnesses with other etiologies. It was observed that there was a wide age range from 3 months to 79 years, equal distribution of the genders affected, and diverse co-infections/comorbidities associated with the purported *Lophomonas* infection. In addition, there are reports of

TABLE 2 Demographic, clinical, diagnosis, treatment, and outcome data of patients (n = 58) putatively diagnosed with *Lophomonas* infection

Variables	Total (58)
Mean age, year (±SD)	38.65 (±22.85)
Sex	
Male	27 (46.55%)
Female	31 (53.45%)
Clinical features	
Cough	41 (70.69%)
Fever	35 (60.35%)
Expectoration	27 (46.55%)
Breathlessness/dyspnea	24 (41.38%)
Chest pain/discomfort	17 (29.31%)
Tachypnea	11 (18.97%)
Hemoptysis	7 (12.07%)
Tachycardia	7 (12.07%)
Immunosuppression	16 (27.59%)
Comorbidities/associated conditions[a]	52 (89.66%)
Diagnosis	
Microscopy	58 (100.00%)
PCR[b]	20 (34.48%)
Sample	
BAL	48 (82.76%)
Sputum	6 (10.35%)
Others[c]	15 (25.86%)
Other microbiological diagnosis[d]	15 (25.86%)
Other microbial therapy	33 (56.89%)
Outcome	
Improvement	35 (60.35%)
Deterioration	3 (5.17%)
Death	6 (10.35%)
Not mentioned	14 (24.14%)

[a]Acute myelocytic leukemia, allergic rhinitis, asthma, breast cancer, bronchitis, bronchomalacia, chronic obstructive pulmonary disease, chronic renal failure, common variable immunodeficiency, Diabetes Mellitus, hypertension, lung adenocarcinoma, lung fibrosis, pneumonia, lung carcinoma, renal cell carcinoma, renal transplant, tuberculosis, scleroderma, and systemic lupus erythematous.
[b]PCR positive in 19 BAL samples (2 of these also positive in sputum) and 1 nasal discharge.
[c]Bronchoscopic brushing, nasal discharge, sinus exudate, surgical piece, and transbronchial biopsy/smear.
[d]Aspergilloma, *Candida* species, cryptococcosis, cytomegalovirus, echinococcosis, *Klebsiella* species, *Pneumocystis jirovecii*, *Pseudomonas aeruginosa*, SARS-CoV-2, and tuberculosis.

Lophomonas causing sinus and urinary tract infections, not included in this analysis as there were no images available (2–5). All 58 cases were identified by microscopy, while PCR was used for the diagnosis in 35% of the reports. There were only two tissue samples obtained in the 58 cases; however, no tissue cytopathology evidence was demonstrated to attribute the disease process to *Lophomonas*. In about 26% of the cases, an alternative microbiological diagnosis was established, such as bacterial pneumonia, tuberculosis, *Pneumocystis jirovecii* pneumonia (PCP), cytomegalovirus, COVID-19, and so on, which can explain the clinical course of the illness. The radiological features of pneumonia in most cases indicate some pneumonia-causing etiology. In about 74% of the reports, however, the diagnostic workup did not yield an alternative diagnosis. It must be noted that even in the best of settings, the etiological agent of pneumonia may remain undiagnosed in more than half of the cases. In the USA, of a cohort of 9,642 patients with a diagnosis of pneumonia over a period of 6 years, 4,432 (46.1%) remained culture-negative (31). Another study from France reported 56% culture-negative pneumonia in 289 non-immunocompromised ICU patients with community-acquired pneumonia (32). In other nations with lesser resources, the incidence of culture-negative pneumonia

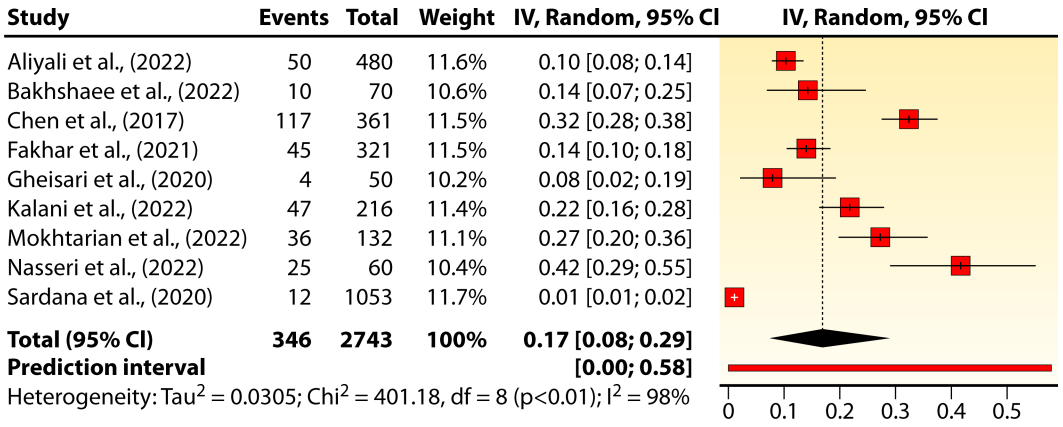

FIG 4 Forest plot for the pooled prevalence of putative *Lophomonas* in patients either by microscopy or PCR.

is expected to be higher. Many respiratory tract pathogens, such as culturable obligate anaerobes, fastidious organisms that require specialized media such as *Mycoplasma pneumoniae*, *Legionella pneumophila*, slow-growing, and unculturable organisms, and viruses are often missed (33). The anaerobic bacteria responsible for respiratory conditions may often be missed due to the stringent sampling and culture requirements (34). The scale of potentially undetected pathogens in pneumonias becomes apparent when advanced methods are applied for detection, and these may include a variety of both culturable and non-culturable respiratory pathogens. In a recent report, when metagenomics was applied on BAL and sputum samples of 292 hospitalized patients suspected to have a lower respiratory tract infection, pathogens were detected in 63 out of 161 (39%) culture-negative samples, and 38 anaerobic bacterial species which did not grow on culture were identified (35). In another study, respiratory pathogens were detected in 40 out of 45 (89%) samples which were culture-negative by shotgun metagenomic sequencing (36). Though there are many caveats to consider while attributing causation of a disease to pathogens with the use of clinical metagenomics using both targeted and non-targeted approaches, namely the problem of background reads from human genomic DNA, the assays not being strictly quantitative, PCR bias, variable rRNA copy numbers, and so on, nevertheless, as highlighted by each of these studies, the limitation of current microbiology diagnostic methodologies that some pathogens tend to remain undetected must be acknowledged, and the patient management plan must follow appropriate guidelines.

Among the 58 patients in our analysis, additional microbial therapy was administered in 56% of the reports, to which the patients were reported to not respond. About 25% of the patients had some form of immunosuppression, and 83% had comorbidities, which could be a possible reason for the lack of therapeutic response. The majority of these cases were reported to have responded to metronidazole, indicating that they could potentially be undiagnosed anaerobic bacterial pneumonias. However, there is no evidence to substantiate these speculations. Notably, there is no evidence of the susceptibility of *Lophomonas* to metronidazole, nor have any attempts been made to determine the susceptibility *in vitro*. Li and Gao in their analysis of 154 cases of *Lophomonas* infection concluded that these reports were likely to be misidentifications of bronchial ciliated epithelial cells, and suggested that the therapeutic effect of metronidazole could be related to the anti-anerobic activity of the drug (8).

The meta-analysis on retrospective and case-control studies estimated a pooled prevalence of 17% with a high heterogeneity indicating that there is much variability in the results of individual studies. The heterogeneity may be due to the differences in study design, type of population, testing characteristics, and so on, however, it may also arise because of the absence of a common confirmatory diagnosis. There are also other assumptions in the *Lophomonas* publications that are not supported by the study

design or findings, for instance, a higher incidence of lophomoniasis in males is due to their increased exposure to the outside environment (37), that there may be an association of the disease with testosterone levels, or that smokers are less affected than non-smokers because smoking damages the normal epithelium, thereby damaging the parasite attachment site, or possibly because cigarette smoke contains substances unfavorable to the survival of the protozoa (37).

Overall, the clinical symptoms, organ systems affected, and associated comorbidities of lophomoniasis are highly heterogeneous, which render these diagnoses at best ambiguous. *Lophomonas* is unlikely to be causing disease in these patients. In such diagnostic quandaries, a rigorous evidence-based approach for detecting alternative etiologies must be applied.

MOLECULAR ANALYSIS

In 2019, a diagnostic PCR for *L. blattarum* was published (9). The new primers were designed against an alignment of a previously published *L. blattarum* short *18S rRNA* sequence from humans (JX020505) and an *18S rRNA* sequence from *L. striata* (JN088049) (9). The specificity of the primers was tested *in silico* only, using Primer-BLAST software. This novel PCR was performed on DNA extracted from the nasal discharge of a patient suffering from acute paranasal sinusitis found to contain putative *Lophomonas* trophozoites (by microscopy) as well as the nasal discharge of a healthy volunteer. Only the patient sample yielded a PCR product of between 200 and 300 bp in size. This product was not sequenced for confirmation of identity as a *Lophomonas* species. No further validation, such as the use of known *Lophomonas* spp. positive controls from cockroaches, tests of analytical specificity against multiple similar protozoa potentially found in human specimens, nor testing of clinical specificity against a panel of known healthy human specimens and proposed *Lophomonas*-infected specimens, were performed. Following the description of this method (9), it has been employed in multiple cases of suspected human *Lophomonas* infection, both in Iran and Thailand.

To investigate the specificity of the Fakhar et al. PCR primers *in silico* and test for possible cross-priming, we first confirmed their potential to amplify *Lophomonas* sequences by nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi.; accessed 25th June 2023) of Fakhar et al.'s primer sequences against *L. striata* (NCBI taxid: 1077219) and *L. blattarum* (NCBI taxid: 1212452). Both primers aligned with the *L. striata* sequences from cockroaches (JN088049 and OP903922) with 100% sequence identity. Both primers also aligned with the *L. blattarum* sequences from cockroaches (OP903921 and OP903923) with 100% sequence identity. We next performed a nucleotide BLAST of the primers against the entire NCBI nucleotide database. Both primers exhibited 100% sequence identity and 100% sequence query cover for human-associated protozoa including *Trichomonas vaginalis* (agent of trichomoniasis, a common STI), *Pentatrichomonas hominis* (a commensal gut inhabitant), *Tetratrichomonas* spp. (some of which inhabit human lungs), and *Trichomonas tenax* (a commensal inhabitant of the human oral cavity and upper respiratory tract). Therefore, the PCR procedure developed by Fakhar et al. is not specific to *L. blattarum*, though it should be capable of amplifying *L. blattarum 18S rRNA* fragments. Furthermore, if these primers are used for diagnosis of a putative *Lophomonas* infection, the PCR product must be sequenced to determine the true source of the amplicon.

To further investigate the specificity of the PCR, we analyzed all 18 of the sequences that have been deposited in GenBank as "*L. blattarum*" from human samples (JX020505; MN243135–MN243136; MZ093069–MZ093079; and OL477421–OL477423 and OL477431). These were all amplified using the Fakhar et al. PCR primers and range in length from 177 to 202 bp, with the exception of JX020505, which was deposited in GenBank prior to the development of the "*L. blattarum*" PCR protocol and is 328 bp in length. We performed a nucleotide BLAST of each of the 18 purported "*L. blattarum*" sequences against the NCBI nucleotide collection. The top match or matches for all were others in the selection of 18 sequences tested. However, three of the sequences also

matched with identities of between 98% and 100% with *P. hominis* (including JX020505), while the rest matched *Tetratrichomonas* spp. with up to 99.01% identity, in many cases representing total sequence differences of only one single nucleotide polymorphism. The 18 "*L. blattarum*" *18S rRNA* sequences were also compared to sequences of *T. tenax 18S rRNA* (NCBI: txid 43075). Some of the "*L. blattarum*" sequences did not meaningfully overlap with published *T. tenax* sequences, but for those that did overlap with 97–100% query cover, the percent sequence identities ranged from 86.67% to 93.31%. This indicates that the published "*L. blattarum*" sequences are unlikely to derive from *T. tenax*.

We also computed the pairwise identities between each of the "*L. blattarum*" sequences from human samples and their closest counterparts in GenBank (*Pentatrichomonas* and *Tetratrichomonas*) and compared them to the pairwise identities between the "*L. blattarum*" sequences and the true *L. blattarum* sequences obtained from cockroaches (11). Sequences JX020505, MN243135, and MN243136 are 98.01–99.09% identical to their top two BLAST hits *P. hominis* OP364969 and KC594038. The other published "*L. blattarum*" sequences, MZ093069–MZ093079, OL477431, OL477421, and OL477422, are 97.30–99.01% identical to their top BLAST hits of various *Tetratrichomonas* spp., AY245117, AY245118, HQ149992, MN309992, and MK801507. Meanwhile, the "*L. blattarum*" sequences from humans are only 79.56–86.21% identical to *L. blattarum 18S rRNA* from cockroaches (11). Therefore, none of the published "*Lophomonas*" sequences from humans are derived from true *Lophomonas*. This conclusion is consistent with a recent phylogenetic analysis, which demonstrated that all "*L. blattarum*" sequences from humans are closely related to either *Tetratrichomonas* spp. or *P. hominis*, while *L. blattarum* and *L. striata* are more closely related to termite symbionts (11).

Comments/conclusions on molecular analysis

The PCR employed for many recent diagnoses of human "*Lophomonas*" infection is not specific to *Lophomonas* spp. It can clearly amplify trichomonad protozoan species, according to studies that used these primers and obtained sequences attributable to *P. hominis* and *Tetratrichomonas* spp., and it is expected to be able to amplify *T. tenax* if present. Therefore, the PCR may simply indicate the presence of known commensals of the human respiratory or gastrointestinal tract, not necessarily the presence of *Lophomonas*. We recommend development of a new PCR assay with primers designed to amplify *Lophomonas* but not trichomonad commensals of humans. This assay should be validated in accordance with well-recognized guidelines such as those by CLSI (38), and the International Organization for Standardization, including testing against confirmed identity isolates of *Lophomonas* spp. from cockroaches, testing against cultured human commensal protozoa, including *P. hominis*, and clinical specificity testing in a population of healthy individuals. Sequence analysis of all amplicons generated in any clinical study to ensure the veracity of any positive results is strongly indicated.

LOPHOMONAS AS A PATHOGEN?

A microbe is typically subjected to rigorous scrutiny before being acknowledged as a true pathogen. The general principles of associating the causation of a disease by a microorganism are laid down by Koch's postulates which require the universal presence of the microbe in the diseased organ, isolation of the microbe from the diseased organ, re-creation of the disease process by the microbe, and re-isolation of the same microbe (39). Although exceptions to Koch's postulates are known, such as the difficult-to-culture organisms like certain viruses, rickettsiae, parasites, and so on, these criteria, nevertheless, serve as guiding principles for determining the pathogen status of microorganisms. Generally, this process includes proof of transmission of the microbial agent to the human host, demonstrating the lifecycle of the agent in the environment, and determining the mode of infection. Upon infecting the human body, almost all microorganisms elicit a specific tissue tropism by attaching to specific receptors of that tissue, which is essential for the establishment of the microorganism in that organ system. Finally, evidence of tissue invasion and disease causation by the microorganism is generated

by elucidating the cytopathological effects in cells, tissues, animal, or human tissues. *Lophomonas* fails to meet any of the criteria for a pathogen. The only criterion it addresses is that it has been putatively detected in diseased individuals; however, the current analysis strongly suggests that both the microscopic and molecular detection of *Lophomonas* in the published literature are erroneous.

There are several gaps in our understanding of the various characteristics of *Lophomonas* as a potential human pathogen. Little is known about its lifecycle and environmental transmission. The published literature suggests that the route of entry of *Lophomonas* in the human body is by inhaling cysts from cockroach feces, after which the trophozoites reside in lower and upper respiratory tracts, however, no experimental evidence exists for any of these suggested phenomena. *Lophomonas* has been reported to affect a variety of tissues, including the bronchi, lung tissue, sinuses, and urinary tract, making the matter of tissue tropism uncertain. In addition, *Lophomonas* has been reported in both the upper and lower respiratory tract, suggesting that it is capable of invading the lung tissue, although there is no evidence to substantiate the same. Whereas the majority of published radiology descriptions of *Lophomonas* infections indicate consolidation-like lung features, there is no evidence of cytopathological effects of *Lophomonas* and tissue pathology features obtained from lung biopsy that can corroborate the role of *Lophomonas* in the disease process. Notably, *Lophomonas* has never been cultured from patient samples, whether identified by PCR or microscopy. These characteristics are essential to establish the diagnosis of a microorganism as the etiological agent of a disease, and their lack in the current literature renders the possibility of *Lophomonas* causing disease in humans unsubstantiated.

CONCLUSION

In this review, we have conducted a systematic analysis of the published literature on *Lophomonas* as a cause of human disease. In the publications, a high heterogeneity in the clinical manifestations and affected organ systems was observed. The diagnostic procedures used to corroborate the identification of *Lophomonas* were insufficient. The few images of acceptable quality available for analysis are variations of host bronchial ciliary epithelial cells and clearly indicate misdiagnoses, while the PCR which has been used in the reports has not been adequately validated for detection of the parasite and has not yet been demonstrated to amplify true *Lophomonas* sequences. Moreover, *Lophomonas* has never been cultured from patient samples. Apart from the putative identification of *Lophomonas* in respiratory samples, there has been no direct demonstration of the parasite's ability to cause pneumonia in humans or animals, and no experimental or tissue evidence of its invasion or immunopathogenicity (*in vitro* or *in vivo*), or its environmental transmission. Therefore, diagnostic laboratories and treating physicians must approach such diagnoses based on microscopy or the currently used PCR with utmost caution. Such misdiagnosis may lead to inappropriate use of antibiotics, particularly metronidazole, which is used for anaerobic bacterial and protozoal infections and can cause long-term consequences due to increased resistance and may potentially compromise patient care and outcomes.

It is notable that the publication of misidentifications can result in the accumulation of confounded scientific literature over the years. The current era of genomics has equipped us with powerful tools such as nucleic acid amplification tests (NAATs), enabling us to identify pathogens rapidly and accurately. However, they must be applied with caution when establishing a diagnosis as they are also susceptible to misinterpretation and misdiagnosis in the absence of rigorous criteria for their validation and clinical application. A misdiagnosis may lead a patient away from the appropriate treatment, resulting in more harm than good. Although NAATs are appealing due to their various useful attributes, they have not replaced classical parasitology (40). Before publishing *Lophomonas* manuscripts and case reports, academic journals must apply rigorous scrutiny and provide the peer reviewers with multiple high-quality images and videos for analysis, bearing in mind that the currently reported PCR alone is insufficient for

the diagnosis. On the other hand, a futuristic approach for resourceful laboratories may be to apply advanced genomic techniques to detect the etiological agents in cases of non-culturable pneumonia (35). Of note, clinical metagenomics is still an emerging approach and not yet standardized for diagnostic use. Nevertheless, when facing diagnostic quandaries due to lack of evidence for the etiologic agent, the patient management plan must follow appropriate guidelines.

Many parasitic diseases are being increasingly reported to be emerging and reemerging in many regions of the world due to globalization, immigration/emigration, environmental changes, and increasing immunosuppression, making it vital to recognize emerging pathogens (41). However, to prove a causal association between the putative microorganisms and the disease, they must undergo rigorous scientific scrutiny. This rigor has not been applied to the identification and reporting of *Lophomonas*, which may be detrimental to both patient care and the scientific literature.

AUTHOR AFFILIATIONS

[1]Department of Medical Parasitology, Postgraduate Institute of Medical Education and Research, Chandigarh, India
[2]School of Life Sciences, Arizona State University, Tempe, Arizona, USA
[3]Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City, Utah, USA
[4]Department of Pathology, University of Utah, Salt Lake City, Utah, USA
[5]Federation University, Melbourne, Australia
[6]Division of Clinical Microbiology, Mayo Clinic, Rochester, Minnesota, USA

AUTHOR ORCIDs

Abhishek Mewara http://orcid.org/0000-0002-1044-6167
Gillian H. Gile http://orcid.org/0000-0003-3955-0942
Blaine Mathison http://orcid.org/0000-0001-9975-941X
Bobbi Pritt http://orcid.org/0000-0003-0261-1326
Richard S. Bradbury http://orcid.org/0000-0002-5524-506X

AUTHOR CONTRIBUTIONS

Abhishek Mewara, Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing | Gillian H. Gile, Data curation, Formal analysis, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing | Blaine Mathison, Conceptualization, Data curation, Formal analysis, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing | Huan Zhao, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review and editing | Bobbi Pritt, Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing | Richard S. Bradbury, Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing

ETHICS APPROVAL

Ethical approval is not required as we utilized secondary data in this study.

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Supplementary tables and figures (JCM00845-23-S0001.docx). Supplementary Tables ST1 to ST5, Supplementary videos SV1 to SV3, Supplementary Fig. 1.
Video S1 (JCM00845-23-s0002.mp4). Video (#1) of motile ciliated respiratory epithelial cells.
Video S2 (JCM00845-23-s0003.mp4). Video (#2) of motile ciliated respiratory epithelial cells.
Video S3 (JCM00845-23-s0004.mp4). Video of motile *Lophomonas blattarum* (courtesy Gillian Gile).

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