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ORIGINAL PAPER

Morphological and Molecular Identification of Emerged *Lophomonas blattarum* Infection in Mazandaran Province, Northern Iran: First Registry-Based Study

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Abstract

Background In the last decade, several cases of bronchopulmonary lophomoniasis (BPL) have been recorded. Little information is available about epidemiological aspects on *Lophomonas* infection among BPL patients. The present study was aimed to investigate the prevalence of *Lophomonas* spp. infection in patients who were referred to the Iranian National Registry Center for Lophomoniasis (INRCL), using morphological and molecular tests.

Subjects and Methods We examined patients enrolled in the INRCL from 2017 to 2019 at the Mazandaran University of Medical Sciences, northern Iran. All bronchoalveolar lavage fluid (BALF) and two nasal discharges of the patients were examined by both microscopic and small-subunit ribosomal RNA (SSU rRNA) PCR methods. To confirm the species of *Lophomonas*, two positive samples were sequenced.

Results In this study, 321 specimens (including 319 BALF and 2 nasal discharges) were microscopically examined. *Lophomonas* spp. was found in 45(14%) ($n=44$ BAL; $n=1$ nasal discharge). The mean age of infected patients was 54.9 ± 17.1 years. The following morphological characteristics were observed in both fresh and Papanicolaou-stained smears to identify *Lophomonas* spp. All microscopically positive specimens were confirmed with genus-specific PCR technique. The obtained sequences were deposited in Gen Bank under the accession numbers (MN243135-36). The BLAST analysis of our two sequences with the only available sequence in the Gen Bank of the Thailand strain of *L. blattarum,* showed identity of 99–100% and 98.51%, respectively.

Conclusion To the best of our knowledge, this is the first registry-based study regarding lophomoniasis worldwide. According to our study, the conventional PCR test is an available and reliable tool for confirming the *Lophomonas* parasite in clinical samples. Moreover, the results confirmed that *L. blattarum* is circulating at least in our region.

Keywords Lophomoniasis · National Registry Center · Microscopic examination · rRNA PCR

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Introduction

Lophomonas parasite is an extracellular flagellated protozoon found in the order Hypermastigida and suborder Lophomonadina. It is accepted as an endocommensal in the intestine of cockroaches such as *Periplaneta americana* (American cockroaches) and *Blattella germanica* (German cockroaches). *Lophomonas* comprise two species: *Lophomonas blattarum (L. blattarum*) and *Lophomonas striata (L. striata*) [1, 2]. The morphological characteristics are as follow: *Lophomonas* spp. range in size from 20 to 60 µ in length and 12–20 µ in width. Their cytoplasm is seen as granular and food phagocytosis. The nucleus is placed as a circular and dark body next to the cilium. Although both *L. blattarum* and *L. striata* have a tuft

of flagella, the body of *L. striata* is tightly folded in its longitudinal axis by striations clearly located on its surface and resembling closely those reported for certain bacteria. Multiplication of the trophozoites is mainly by binary fission [3]. Encysted forms have been described and fully formed cysts are spherical or sometimes oblong and surrounded by a homogeneous membrane [4].

The first case of pulmonary *L. blattarum* infection was reported in China in 1993 [5]. Actually, the details of transmission of this neglected and emerging protozoan disease still remain a mystery. But the acceptable route of transmission is inhalation of infected air/dust with a cyst of the parasite. It can cause infections in a variety of tissues and organs, including the sinuses, lungs and the rest of the respiratory tract. These respiratory tract infections are difficult to differentiate from other common causes, e.g. viral, bacterial or fungal, by clinical manifestations only. Pulmonary lophomoniasis is a treatable infection and metronidazole is the drug of choice [6]. Several cases of human infection have been reported from Spain [7, 8], Peru [9], Turkey [10], India [11], Mexico [12] and Iran [13]. Most of the cases reported in Iran were children [13–16].

Patients with respiratory asthma, allergic rhinitis, immune deficiency, especially AIDS, organ transplantation, cancer and under treatment with corticosteroids are more susceptible to being infected by *Lophomonas* spp. protozoa [10, 17]. Little is known about the epidemiological aspects of *Lophomonas* infection.

German cockroache *(Blattella germanica*) as a common domestic pest has been reported from all parts of Iran [18]. Based on our recent study, *Lophomonas* spp. was detected in the gut of German cockroaches trapped in hospitals for the first time in Mazandaran Province, Sari, northern Iran [19]. This study indicates that at least one of the sources of infection in this region may be close contact to infected German cockroaches, which may pose a risk to patients admitted to these hospitals [19].

Considering the lack of data on the status of lophomoniasis among patients in Mazandaran Province, northern Iran, the present study aimed to determine the prevalence of *Lophomonas* spp. infection and survey epidemiological aspects also in patients who were referred to the Iranian National Registry Center for Lophomoniasis (INRCL), with chronic respiratory disorders, for the first time, using morphological and molecular techniques.

Subjects and Methods

Study Area and Sample Collection

This descriptive analytical study was performed in the pulmonary clinic at Imam Khomeini Hospital, Mazandaran Province, Sari, northern Iran, from December 2017 to July 2019. The province is located in the north of Iran (53°6′ E, 36°23′ N). From the geographical point of view, the province is divided into two parts, i.e. the coastal plain and the mountainous area. The central zone of the province has humid weather and also has an annual mean rainfall of 977 mm [20]. The bronchoalveolar lavage fluid (BALF) specimens of the patients submitted to the Iranian National Registry Center for lophomoniasis (INRCL) at Mazandaran University of Medical Sciences, Sari, Iran, were used for detecting *Lophomonas* spp. A detailed questionnaire was conducted, and physical examinations were performed. Clinical symptoms and signs, gender, age, living area, past medical history and type of specimen were recorded.

Fiberoptic Bronchoscopy (FOB)

Flexible fiberoptic bronchoscopy (FOB) examination was performed for all patients who are suitable candidates for bronchoscopy. A BALF specimen was collected by wedging the tip of the bronchoscope into the nondependent lobes, in particular, the middle lobe of the right lung and the lingula of the left lung. The lobe used for BALF collection was determined by the images of the lesion with the greatest radiologic abnormality. About 5–20 mL of sterile normal saline (with a total volume of 1 mL/kg) was instilled, divided into 5–20 aliquots, for 2–4 times. Gentle manual suction was applied to retrieve the saline. BALF was collected in sterilized containers, and 2–5 mL of BALF was submitted to the laboratory of INRCL at Imam Khomeini hospital within 1–3 h at room temperature.

Microscopic Examination

319 BALF and two nasal discharge specimens were collected and each one centrifuged at 2000 rpm for 5 min and the sedimentation was smeared onto a glass slide and examined under a light microscope for the presence of *Lophomonas* spp. We have also considered the *Lophomonas* density estimation under a light microscope. Based on our experience in INRCL, an innovative severity index (SI) for *Lophomonas* infection was scored as mild to severe parasite density by counting parasites per high power microscopic fields (HPF) (X400). Accordingly, the mild density was defined as 1–10 parasites/100HPF, moderate as 1–10 parasites/10HPF, and 1–10 parasites/HPF were considered as severe (Unpublished data). Also, all positive smears were stained by Papanicolaou stain to investigate more details such as flagella, nucleous, etc.

Molecular Examination

DNA Extraction

Accordingly, 200 µL of the specimen was homogenized in 200 µL of digestive buffer consisted of 50 mM Tris–HCl (pH 7.6), 1 mM EDTA and 1% Tween 20, to which 20µL of the proteinase K solution (containing 20 mg of the enzyme/mL) was added. Afterward, the homogenized solution was incubated at 45 °C overnight. Next, 200 µL of phenol: chloroform: isoamyl alcohol (25:24:1) solution was added to the latter homogenate. After being shaken vigorously, the solution was centrifuged at 14,000×*g* for 15 min. The supernatant was poured into a fresh microtube to which 400 µL of the cold absolute ethanol was added and kept at − 20 °C for 2 h. Afterward, 200 µL of 70% ethanol was added to the precipitate, centrifuged, and the precipitate was suspended in 50 µL of double-distilled water and stored at 4 °C until use [21].

Genus-Specific rRNA PCR Amplification

The PCRwas set up in a total volume of 25 µL, which included 12.5 µL of the Master Mix (Fermentas, Inc.), 1 µL of each primer forward (F) (5′-GAG AAG GCG CCT GAG AGA T- 3′) and reverse (R) (5′-ATG GGA GCA AAC TCG CAGA- 3′) specific primers that was planned from small-subunit ribosomal RNA (SSU rRNA) [21], 5 µL of the extracted DNAand 5.5 µL of distilled water. Thirty-five cycles were performed in a thermocycler (Corbett Research, Sydney, Australia) with initial denaturation at 94 °C for 2 min, followed by 40 cycles of 94 °C for 1 min, 57 °C for 1 min and 72 °C for 1 min, and then a final extension at 72 °C for 3 min. Subsequently, 6 µL of the PCRproducts was analyzed by electrophoresis on 1.5% (w/v) agarose gel in Tris–borate-EDTA (TBE) buffer. The electrophoresed PCR products bands were observed using UV transillumination after staining with SYBR® Safe Stain (Invitrogen®). *Lophomonas* DNA obtained in our previous study [5] and water without DNA were included in all test for positive and negative control respectively. The amplicons using Sanger sequencing technology was sequenced and then edited with BioEdit software (v.7.2).

Data Analysis

We used frequency and percent to describe qualitative variables, mean and standard deviation for quantitative variables. To assess relationship between each variables and positive samples odds ratio and regression test with 95% confidence interval (95%CI) were applied. The p-value less than 0.05 were regarded as statistically significant. All statistical analysis was performed in IBM SPSS version 23.

Results

In this study, 321 specimens (including 319 BALF and 2 nasal discharges) were microscopically examined. *Lophomonas* spp. were found in 45 (14%) ($n=44$ BAL; $n=1$ nasal discharge). The mean age of infected patients was 54.9 ± 17.1 years (see Table 1). The following morphological characteristics were observed in both fresh and Papanicolaou-stained smears to identify *Lophomonas* spp.: the trophozoite was pyriform in shape and had a tuft of several flagella on the apex (Fig. 1). Moreover, according to SI scoring, most of the (39/45) positive specimens significantly had mild density severity ($P=0.001$).

A total of 45 positive subjects, 27 examined males, and 18 were female. There was a significant relationship between male and *Lophomonas* spp., infection ($P=0.002$). Out of 18 females, 16 were housewives and 2 were others. Occupation and *Lophomonas* infection in females showed significant correlations ($P=0.03$). There was no correlation between occupation and *Lophomonas* infection in males ($P=0.68$). Moreover, all of the patients had a history of contact with cockroaches in indoor places. The majority of patients' symptoms were prolonged cough, hemoptysis and dyspnea. Most patients (86.7%, 39/45) were referred to the INRCL during the fall and winter seasons than other ones.

Additionally, 33.3% (3 of 9) of patients with a history of tuberculosis were also positive for *Lophomonas* infection (see Table 1). No correlation was found between *Lophomonas* infection and underlying disease ($P>0.05$). In all enrolled patients, FOB findings showed mild to moderate mucosal edema and hyperemia (see Fig. 2). All patients were also properly treated with metronidazole.

Also, all microscopically positive specimens were confirmed with the genus-specific-SSU rRNA PCR technique (Fig. 3). Moreover, two high-quality BALF samples with sharp and non-smear bands were sequenced to confirm PCR results and deposited in the GenBank by BanKit (Accession Numbers: MN243135–36). The identity and query coverage of theses isolates in comparison with only available *L. blattarum* that was the first report of bronchopulmonary infection caused by *L. blattarum* in Thailand (Accession Numbers: JX020505.1), were 99–100% and 98.51%, respectively.

Discussion

Little information is known about several aspects of lophomoniasis, such as clinical features and laboratory findings worldwide. In the present study, *Lophomonas* infection, for the first time, was found in 14% of the sampled population in northern Iran. The high prevalence of lophomoniasis in Mazandaran Province could be attributed to the warm and

Table 1 Characteristics of patients enrolled in this study

Variable	No. of examined	No. of positive	OR (95%CI)	*P*-value
Gender				
Male	206	27	2.97 (1.67–5.12)	0.002
Female	115	18	Reference	
Age group				
10–20	5	2	4.25(0.67–27.10)	0.13
21–30	13	3	1.91 (0.49–7.57)	0.38
31–40	27	4	1.0 (0.37–2.71)	0.99
41–50	44	6	0.86 (0.31–1.19)	0.72
51–60	92	11	1.91(0.49–7.57)	0.38
> 60	140	19	Reference	
Living area				
Urban	211	31	1.18 (0.60–2.32)	0.63
Rural	110	14	Reference	
Past medical history				
TB	9	3	3.0 (0.23–37.67)	0.39
Lung cancer	9	1	0.75 (0.04–14.5)	0.84
Transplantation	2	1	0.0 (0.18–198.1)	0.31
Diabetes mellitus	13	2	1.10 (0.08–14.67)	0.94
Asthma	7	1	Reference	
Infection severity				
Mild	45	39	10.0 (0.98- 210.9)	0.001
Moderate	45	3	0.1 (0.12–18.5)	
Severe	45	3	Reference	
Specimen				
Nasal	2	1	6.5 (38.1–101.2)	0.20
BAL	319	44	Reference	
Total	321	45		

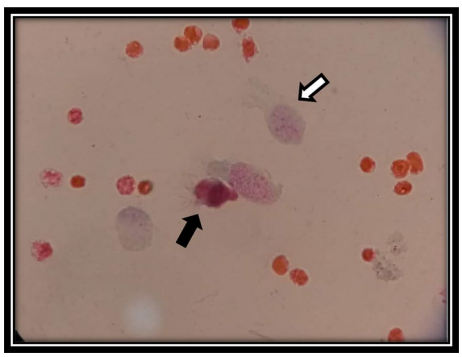

Fig. 1 *Papanicolaou staining of bronchoalveolar lavage fluid showing L.blattarum* trophozoite (black arrow) and bronchial epithelial cell (white arrow) (magnification × 40)

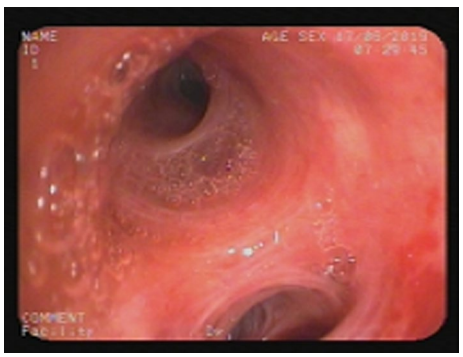

Fig. 2 Brochoscopic view of left upper division (LUD) in a patient with chief complaint of hemoptysis and ground-glass opacity in the left upper lobe on computed tomography scan images. After irrigation with cold normal saline, mild mucosal edema and hyperemia were evident

humid weather, which promotes the growth of the vector of *Lophomonas*, specifically cockroaches and house dust mites [2, 22, 23]. In addition, contact with family members may increase the risk of transmission of *Lophomonas* infection. *Lophomonas* transmission via airborne is a possibility for this parasite. Every year, a large number of tourists from all over Iran and neighboring countries trip to Mazandaran Province, which may pose a risk to the tourists during

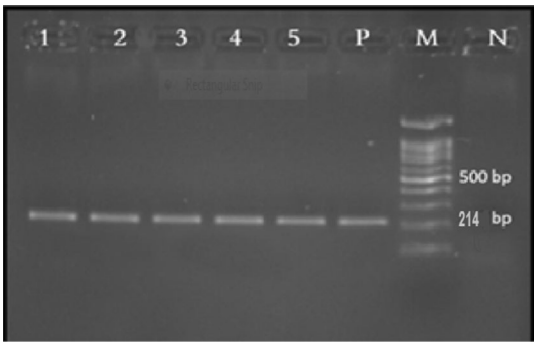

Fig. 3. 1.5% agarose gel electrophoresis, showing the 214 bp band of PCR product, confirming *Lophomonas* spp. in the patient specimens. M: 100-bp DNA ladder; 1–5: PCR products of the patient specimens; *N* negative sample

vacation. In our study, the majority of patients were males. These results are in good agreement with other case studies. Based on previous studies, *Lophomonas* infection has been frequently reported in males [5, 10, 16, 24].

Despite the fact that Ghafarian et al. (2018) found that there is a high prevalence of lophomoniasis in girls. It's noted that this study was conducted only on under 18-year old children in Mashhad, eastern Iran [14]. Males have a higher susceptibility to many parasitic infections compared to women such as *Plasmodium, Leishmania* and *Entamoeba histolytica.* This can be explained by sex differences in immune function with circulating steroid, genetic and behavioral differences [25–28].

Among 45 patients, 31 (68.8%) were inhabitants in urban areas and the rest of them in rural areas. Even though our results revealed no significant difference between *Lophomonas* infections and inhabited areas ($P=0.63$), the high population of cockroaches in urban areas can be attributed to the high prevalence of disease in these areas [29].

Surprisingly, in our study, all patients had a history of contact with cockroaches. Previous studies have reported isolation of the parasites in cockroaches and house dust mites indoor [30, 31]. Additionally, our recent study showed 1.7% of the trapped German cockroaches from different parts of hospitals in Mazandaran Province, Sari (place of the current study) were infected with Lophomonas spp. [19].

There are several possible explanations for the high prevalence of lophomoniasis in our study. For instance, a high population of cockroaches and house dust mites has been reported in the North of Iran [22, 23].

Several allergic reactions among the general human population provide evidence of the presence of cockroaches/mites. The main clinical manifestations of *Lophomonas* spp. infection are not based on clinical signs. Due to the similarity of clinical symptoms with other respiratory diseases, it is difficult to diagnose and/or differentiate from other conditions [3]. However, our data showed clinical findings of the patients were similar to other studies, including, hemoptysis, prolonged cough, wheeze and dyspnea [5, 6, 32, 33]. In this regard, based on available data and our population-based study in INRCL, since most patients suffer from hemoptysis, we recommend the "pink cough" term for lophomoniasis. Hence, this term can be appointed for bronchopulmonary lophomoniasis within publications regarding the disease in future.

In the present study, the majority of lophomoniasis were found during cold weather months (fall and winter). The high prevalence of the disease during these seasons may be attributed to the relatively long patent period of the infection among patients, who were frequently infected during summer, as a season with a high population of German cockroaches. On the other hand, inhaling cold air, which causes to produce proinflammatory substances and epithelial injury. Other effects of cold air contact include an increase in BAL fluid granulocytes, loss of ciliated epithelium, thickening of the lamina propria with increased concentrations of inflammatory cells, hyper responsiveness, and airway obstruction [34].

In the present study, although there was no correlation found between *Lophomonas* infection and underlying disease, the highest rate (33%) of co-morbidity was found among patients with tuberculosis. This report substantiates previous findings in the literature [32, 34, 35]. Consequently, since *Lophomonas* is an opportunistic parasite, it should be examined in respiratory secretions of patients with resistant tuberculosis treatment. Most parasitic agents in different organs that co-infect with *Mycobacterium tuberculosis* improve intolerance to antibacterial therapy and deteriorate the prognosis, according to a systematic review [35]. In addition, since tuberculosis and lophomoniasis have some clinical similarity, *Lophomonas* infection should be ruled out in patients suspected of having tuberculosis. However, *Lophomonas* infection may influence the patient's immune response and the course of the disease.

Given that there are no specific signs and symptoms for lophomoniasis [5, 10, 14, 16], laboratory findings, particularly PCR test, are critical for identifying this emerging disease. In this regard, microscopic examination, particularly wet mount, is routinely used as the most available method for diagnosis of *Lophomonas* parasite. Several morphological features are common between *Lophomonas* and lung epithelial cells such as creola bodies (small groups of ciliated bronchial cells) and ciliocytophthoria (detached ciliary tufts with cytoplasmic remnants). The presence of round and oval shapes in both, size similarities in the range of 15–40 µ, and motions of the cilia and flagella in the parasite have made light microscope differentiation difficult. More than 50 irregular arranged flagella with unsynchronized rhythmic, granular cytoplasm and unclear nucleous which are specific

for *Lophomonas*. While lung epithelial cells were recognized by synchronized and rhythmic motivation of cilias, terminal bar, terminal nucleous [3, 36, 37].

In our study, Papanicolaou and Giemsa stains were used to show morphological details of *Lophomonas* spp. Several stains, including Hematoxylin/Eosin, Giemsa, and Papanicolaou, as well as trichrome stain, have been recommended in the literature. However, this is subject to each laboratory's availability and/or shipment to a referral center with more preparation for parasite identification [3, 5, 14, 21, 38]. However, a study compared several staining methods to show morphological details of *Lophomonas* spp. on one BAL specimen. Based on the results, Trichrome stain had the best and Papanicolaou and Giemsa stains had medium quality [36].

Based on our experience at LNRC, the sensitivity and specificity of the *Lophomonas* wet mount examination is relatively low. One factor that can reduce the sensitivity of the wet mount is delays between specimen collection and microscopic examination. In addition, suboptimal specimen storage or transportation conditions further reduce parasite motility and thus wet mount sensitivity. Well trained microscopists for accurate identification have a great role in increasing the sensitivity of the diagnostic method. However, the advantages of this method are its available, rapid, simple and low cost [39].Consequently, the molecular-based diagnosis is at least necessary to confirm *Lophomonas* spp. In the present study, 45 specimens that were positive by the wet mount method were confirmed by the PCR test. Since PCR is an expensive and less accessible method further studies on microscopic examination should be undertaken.

To date, only one study of this protozoan has been diagnosed by PCR method [21]. The high sensitivity and specificity of the PCR has persuaded researchers to recommend a molecular method for the detection of lophomoniasis [3, 10, 36, 40]. It can detect organisms in latent infections, can identify organisms that cannot be cultured, avoids false positives, can detect organism before antibodies or antigens are produced, can detect organisms in tissue and vectors [39]. So, the molecular-based diagnosis was performed in the current study to confirm *Lophomonas* spp. It should be noted that the PCR test is currently available at INRC for detecting the parasite in different clinical samples.

Conclusion

To the best of our knowledge, this is the first registry population-based study regarding lophomoniasis. According to our study, the conventional PCR test is an available and reliable tool for confirming the *Lophomonas* parasite in clinical samples. Moreover, the results confirmed that *L. blattarum* is circulating at least in our region. Accordingly, further studies are recommended for developing a specific PCR for discriminating the parasite species such as multiplex-PCR as well as sequencing analysis of the high number of clinical samples for detecting *Lophomonas* species/genotyping. As a whole, our data provides unique information about different epidemiological evidence about BPL in the world.

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Declarations

Conflict of interest The authors pronounce that there are no conflicts of interest.

Ethical approval The current study was approved by the Ethical Committee of the Faculty of Medicine, Mazandaran University of Medical Sciences, Iran (IR.MAZUMS.IMAMHOSPITAL.REC.1398.002).

Informed consent All participants wrote the informed consents for participating in this study.

References

1. Gile GH, Slamovits CH (2012) Phylogenetic position of *Lophomonas striata* bütschli (parabasalia) from the hindgut of the cockroach *periplaneta americana*. Protist 163(2):274–283. https://doi.org/10.1016/j.protis.2011.07.002
2. Gullan PJ, Cranston PS (2014) The insects: an outline of entomology. Wiley, Hoboken
3. Martinez-Giron R, van Woerden HC (2013) *Lophomonas blattarum* and bronchopulmonary disease. J Med Microbiol 62(11):1641–1648. https://doi.org/10.1099/jmm.0.059311-0
4. Rr KUDO (1931) Handbook of protozoology, charles c. Thomas Springfield, Illinois
5. Xue J, Li Y-L, Yu X-M, Li D-K, Liu M-F, Qiu J-F, Xue J-J (2014) Bronchopulmonary infection of *Lophomonas blattarum*: a case and literature review. Korean J Parasitol 52(5):521
6. Ribas A, Martínez-Girón R, Ponte-Mittelbrum C, Alonso-Cuervo R, Iglesias-Llaca F (2007) Immunosupression, flagellated protozoa in the human airways and metronidazole: observations on the state of the art. Transpl Int 20(9):811–812. https://doi.org/10.1111/j.1432-2277.2007.00521.x
7. Martínez-Girón R, Doganci L (2010) *Lophomonas blattarum*: A bronchopulmonary pathogen. Acta Cytol 54(5):1050–1051
8. Martínez-Girón R, Ribas A, Astudillo-González A (2007) Flagellated protozoa in cockroaches and sputum: the unhygienic connection? Allergy Asthma Proc 28:608–609. https://doi.org/10.2500/aap2007.28.3042
9. Zerpa R, Ore E, Patiño L, Espinoza Y (2010) *Lophomonas* sp. In respiratory tract secretions in hospitalized children with severe lung disease. Rev Peru Med Exp Salud Publica 27(4):575–577. https://doi.org/10.1590/s1726-46342010000400013
10. Kilimcioglu AA, Havlucu Y, Girginkardesler N, Çelik P, Yereli K, Özbilgin A (2014) Putative bronchopulmonary flagellated protozoa in immunosuppressed patients. Biomed Res Int. https://doi.org/10.1155/2014/912346

11. Verma S, Verma G, Singh DV, Mokta J, Negi RS, Jhobta A, Kanga A (2015) Dual infection with pulmonary tuberculosis and *Lophomonas blattarum* in india. Int J tuberc Lung Dis Off J Int Union Against Tuberc Lung Dis 19(3):368. https://doi.org/10.5588/ijtld.14.0513
12. Saldaña NG, Mendoza FJO, Larrauri FR, Trujillo DMG, Montoya EV, De La Garza EA, Olguín HJ (2017) Bronchopulmonary infection by *Lophomonas blattarum* in a pediatric patient after hematopoietic progenitor cell transplantation: First report in mexico. J Thorac Dis 9(10):E899. https://doi.org/10.21037/jtd.2017.09.19
13. Berenji F, Parian M, Fata A, Bakhshaee M, Fattahi F (2016) First case report of sinusitis with *Lophomonas blattarum* from iran. Case Rep Infect Dis. https://doi.org/10.1155/2016/2614187
14. Ghafarian N, Bakhtiari E, Berenji F, Nakhaei M, Nakhaei B, Jamali-Behnam F, Sayedi J (2018) The study of *Lophomonas blattarum* infection in children with respiratory symptoms: a descriptive clinical study in north east of Iran. Int J Pediatr 6(6):779–802. https://doi.org/10.22038/IJP.2018.29199.2555
15. Mirzazadeh F, Berenji F, Amini M, Salehi M, Shamsian A, Fata A, Parian M, Nakhaei M, Ghofraniha L (2017) *Lophomonas blattarum* in asthmatic patients and control group. J Res Med Dent Sci 5(5):1–5
16. Talebian M, Berenji F, Amini M, Sayedi SJ, Shamsian A, Afzalaghaee M, Fata A, Parian M, Nakhaei M, Farash BRH (2018) A study about clinical symptoms and laboratory signs of adult and pediatric patients with *Lophomonas blattarum*. J Res Med Dent Sci 6(1):312–317
17. Martínez-Girón R, Esteban J, Ribas A, Doganci L (2008) Protozoa in respiratory pathology: a review. Eur Respir J 32(5):1370–1354. https://doi.org/10.1183/09031936.00022008
18. Salehzadeh A, Tavacol P, Mahjub H (2007) Bacterial, fungal and parasitic contamination of cockroaches in public hospitals of hamadan, Iran. J Vector Borne Dis 44(2):105
19. Motevalli-Haghi SF, Shemshadian A, Nakhaei M, Faridnia R, Dehghan O, Shafaroudi MM, Kelarijani MN, Nikookar SH, Kalani H, Fakhar M (2021) First report of *Lophomonas* spp. In german cockroaches (blattella germanica) trapped in hospitals, northern Iran. J Parasit Dis. https://doi.org/10.1007/s12639-021-01381-2
20. Mahjouri E (2001) Historical geography of Mazandaran Province. Geographical Organization Press, Tehran
21. Fakhar M, Nakhaei M, Sharifpour A, Kalani H, Banimostafavi ES, Abedi S, Safanavaei S, Aliyali M (2019) First molecular diagnosis of lophomoniasis: the end of a controversial story. Acta Parasitol. https://doi.org/10.2478/s11686-019-00084-2
22. Ghaffari J, Khademloo M, Saffar M, Rafiei A, Masiha F (2010) Hypersensitivity to house dust mite and cockroach is the most common allergy in north of Iran. Iran J Immunol 7(4):234–239
23. Ghaffari J, Mohammadzadeh I, Khalilian A, Rafatpanah H, Mohammadjafari H, Davoudi A (2012) Prevalence of asthma, allergic rhinitis and eczema in elementary schools in sari (Iran). Casp J Int Med 3(1):372
24. Liu J, Li S, Li H, Fan Y, Yang H, Xu H, Shen Y, Zhao S (2017) Bronchopulmonary disease caused by flagellated protozoa infection in 15 Chinese children. Pediatr Infect Dis J 36(4):392–397. https://doi.org/10.1097/inf.0000000000001455
25. Bernin H, Lotter H (2014) Sex bias in the outcome of human tropical infectious diseases: Influence of steroid hormones. J Infect Dis 209(suppl_3):S107-113. https://doi.org/10.1093/infdis/jit610
26. Fischer J, Jung N, Robinson N, Lehmann C (2015) Sex differences in immune responses to infectious diseases. Infection 43(4):399–403. https://doi.org/10.1007/s15010-015-0791-9
27. Klein SL (2004) Hormonal and immunological mechanisms mediating sex differences in parasite infection. Parasite Immunol 26(6–7):247–264. https://doi.org/10.1111/j.0141-9838.2004.00710.x
28. Muenchhoff M, Goulder PJ (2014) Sex differences in pediatric infectious diseases. J infect Dis 209(suuppl_3):S120-126. https://doi.org/10.1093/infdis/jiu232
29. Nasirian H (2017) Infestation of cockroaches (insecta: Blattaria) in the human dwelling environments: a systematic review and meta-analysis. Acta Trop 167:86–97. https://doi.org/10.1016/j.actatropica.2016.12.019
30. Holgate ST (2010) Disruption of airway epithelium in asthma pathogenesis: are protozoa responsible? Proc Am Thorac Soc 7(2):161. https://doi.org/10.1513/pats.7.2.161a
31. Yang J-X, Tang Y-Y, Fang Z-M, Tong Z-Z, Li Y-L, Wang T (2014) Investigation on *Lophomonas blattarum* infection in periplaneta americana in wuhan city. Zhongguo ji sheng chong xue yu ji sheng chong bing za zhi = Chin J Parasitol Parasit Dis 32(2):161–162
32. Martínez-Girón R, Van Woerden HC (2014) Bronchopulmonary lophomoniasis: emerging disease or unsubstantiated legend? Parasit Vectors 7(1):284. https://doi.org/10.1186/1756-3305-7-284
33. Tyagi R, Anand KB, Teple K, Negi RS (2016) *Lophomonas blattarum* infection in immunocompetent patient. Lung India Off Organ Indian Chest Soc 33(6):667. https://doi.org/10.4103/0970-2113.192867
34. D'Amato M, Molino A, Calabrese G, Cecchi L, Annesi-Maesano I, D'Amato G (2018) The impact of cold on the respiratory tract and its consequences to respiratory health. Clin Transl Allergy 8(1):1–8. https://doi.org/10.1186/s13601-018-0208-9
35. Li X-X, Zhou X-N (2013) Co-infection of tuberculosis and parasitic diseases in humans: a systematic review. Parasit Vectors 6(1):1–12. https://doi.org/10.1186/1756-3305-6-79
36. Alam-Eldin YH, Abdulaziz AM (2015) Identification criteria of the rare multi-flagellate *Lophomonas blattarum*: comparison of different staining techniques. Parasitol Res 114(9):3309–3314. https://doi.org/10.1007/s00436-015-4554-4
37. Li R, Gao ZC (2016) *Lophomonas blattarum* infection or just the movement of ciliated epithelial cells? Chin Med J 129(6):739. https://doi.org/10.4103/0366-6999.178025
38. Wang Y, Tang Z, Ji S, Zhang Z, Chen J, Cheng Z, Cheng D, Liu Z, Li L (2006) Pulmonary *Lophomonas blattarum* infection in patients with kidney allograft transplantation. Transpl Int 19(12):1006–1013. https://doi.org/10.1111/j.1432-2277.2006.00380.x
39. Morgan U, Thompson R (1999) Molecular detection of parasitic protozoa. Parasitology 117(07):73–85. https://doi.org/10.1017/s0031182099004102
40. Martínez-Girón R, van Woerden HC, Doganci L (2011) *Lophomonas* misidentification in bronchoalveolar lavages. Int Med (Tokyo, Japan) 50(21):2721–2723. https://doi.org/10.2169/internalmedicine.50.5878