

Review

Lophomonas blattarum and bronchopulmonary diseaseRafael Martínez-Girón¹ and Hugo Cornelis van Woerden²

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The natural habitat of the multiflagellate protozoon *Lophomonas blattarum* is as an endocommensal in the hindgut of insects such as cockroaches. However, it also causes bronchopulmonary disease in humans. The aim of this paper was to review the literature on this organism in the context of respiratory disease. The biology epidemiology, route of transmission, pathogenic mechanisms and diagnosis methods are also described. A total of 61 cases were identified in the literature. The majority of these reports were from China, with some cases from Peru and Spain. Most cases were adult males, although paediatric cases were reported in Peru. Clinical presentation was non-specific, including symptoms such as fever, cough and breathless. Antiprotozoal therapy was generally effective.

Introduction

Clinically significant pulmonary protozoal infections are increasingly being recognized (Vijayan, 2009). This rise may in part be due to an increasing proportion of the population having compromised immune function. Compromised immune function in the respiratory tract can be due to infection with the human immunodeficiency virus, long-term use of immunosuppressive therapies, cancer, systemic illness, the ageing process, or chronic respiratory pathology. A proportion of the rise in protozoal infections is due to the subgroup of flagellates (Martínez-Girón *et al.*, 2008a). This paper focuses on one of these, a multiflagellate protozoon called *Lophomonas blattarum*.

L. blattarum is a rare but potentially important cause of bronchopulmonary infection and respiratory symptoms (Yao, 2008; Wu & Liu, 2010; Zhang *et al.*, 2011). The organism has been also reported in adult patients with bronchopneumonia, paediatric in-patients with severe pulmonary disease (Zerpa *et al.*, 2010), and adult patients with asthma (Martínez-Girón *et al.*, 2007; Martínez-Girón & Doganci, 2010).

The identification of this protozoon in human samples has been based on the identification of morphological features under light microscopy using fresh and stained samples from the airways including sputum, bronchoalveolar lavages, bronchial brushings and tracheal aspirates. Multiflagellate protozoa are difficult to differentiate from ciliated bronchial epithelial cells, and misidentification under light microscopy is a significant risk (Martínez-Girón *et al.*, 2011). This risk should be reduced in the future with the development of molecular methods of identification.

L. blattarum has not been previously summarized in a comprehensive review. The purpose of this paper is, therefore, to review available literature on *L. blattarum* and to examine this protozoon as an example of an insect endocommensal, where the shared habitat of humans and insects may have wider significance for bronchopulmonary disease.

This literature review was based on extensive searching of PubMed and Google Scholar, which is now one of the most comprehensive online databases, using the search term *Lophomonas*. The references quoted in identified papers were also reviewed to identify additional case reports.

The searches of PubMed and Google Scholar produced 16 and 252 results, respectively, (excluding patents and secondary citations) as of 28 December 2012. The literature identified is summarized below under the headings of: taxonomy and biology, morphology, culture, epidemiology, clinical symptoms diagnosis and treatment. The conclusions also contain some speculation on possible links between *Lophomonas* in sputum and in insects, and the possibility of transmission from insects to humans.

Taxonomy and biology

In recent classifications of unicellular eukaryotes including protozoa and other protists (Adl *et al.*, 2005), *Lophomonas* is described as belonging to the supergroup *Excavata*, first rank *Parabasalida*, second rank *Cristamonadina*. *Lophomonas* are anaerobic multiflagellate parabasalids and comprise two species: *L. blattarum* and *Lophomonas striata*. Both are endocommensals in the hindgut of certain arthropods such as termites and omnivorous roaches (Strand & Brooks,

1977), and have also been described in the faeces of certain birds, such as bustards (Silvanose *et al.*, 1999). *L. blattarum* has been observed in the hindguts of several species of cockroach including *Blatta orientalis* (Kudo, 1926b; Semans, 1941), *Periplaneta americana* (Hoyte, 1961a), and *Blattella germanica* (Tsai & Cahill, 1970). The role that flagellate protozoa play in the hindgut of the cockroaches seems to be one of facilitation of digestion of nutrients, especially the digestion of lignocellulose (Trager, 1932; Wharton *et al.*, 1965).

As endocommensals, these protozoa use part of the food material which could be used by the host, but they do not normally invade the host's tissues. This role is similar to that of other micro-organisms, such as the ciliated protozoon *Nictotherus ovalis* (Gijzen & Barugahare, 1992) and a range of bacteria and fungi (Cruden & Markovetz, 1979; Martin, 1983; Schauer *et al.*, 2012; Gibson & Hunter, 2009).

Morphology

The morphological characteristics of *L. blattarum* under light microscopy are well described (Brugerolle & Lee, 2000). The protozoon appears as round-ovoid or piriform in shape (only the smallest forms possess the more spherical shape), and measures 20–60 µm in length and 12–20 µm in width. The cytoplasm looks granular, with some phagocytosed food particles. At one of the poles (the apical zone) the protozoon has a tuft of numerous flagella with an irregular orientation (Fig. 1a, b). The outer flagella of the tuft are smaller and separate, and vibrate freely in the surrounding fluid medium. Occasionally, the nucleus may be seen as a round dark body situated a short distance under the insertion of the flagella (Bütschli, 1879).

Multiplication of the trophozoites is mainly by binary fission. Encysted forms have been described (Kudo, 1931), and fully formed cysts are spherical or sometimes oblong and surrounded by a homogeneous membrane. In *L. striata*, the nucleus of the cyst undergoes division and forms two daughter nuclei (Kudo, 1926c). Lucas (1928) described excystation of *L. striata*, emerging as a double organism from a small aperture in the cyst wall in the hindgut of cockroaches such as *P. Americana* and *Blatta orientalis*. In his paper he maintains that excystation occurs in both *L. striata* and *L. blattarum*.

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 (Beams *et al.*, 1960).

Under the light microscope and in sputum samples, in both fresh and stained smears by the Papanicolaou method and Wheatley's trichomic stain, the morphological features of *L. blattarum* are clear. Two pathognomonic morphological characteristics of ciliated epithelial fragments were used to differentiate them from flagellated protozoa: the observation of a round-oval nucleus at the basal end of the cell (if a nucleus was present); and a marked terminal bar at the apical end of the cell with regular, unidirectional cilia inserted into the terminal bar (Figs 2 and 3).

In the natural environment, *Lophomonas*' food consists of fluid matter present in the colon of the host. The movements of the flagella create an alimentary flow which wafts this food towards the cell.

Electron microscopy studies of the ultrastructure of flagellate protozoa have shown some peculiar cytoplasmic structures

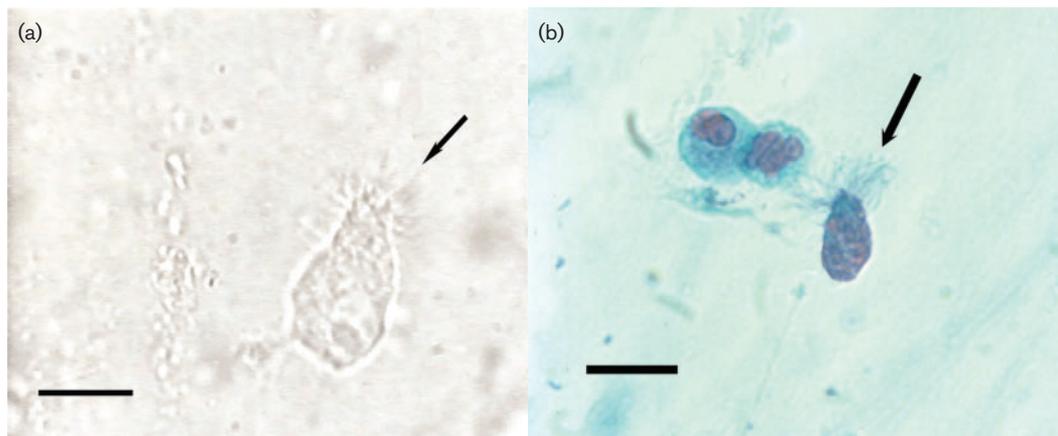


Fig. 1. (a) Flagellate protozoon observed in a fresh sample from a spontaneous expectoration. Note the piriform shape, granular cytoplasm with a large vacuole and the characteristic tuft of numerous and irregular flagella at the apical end (black arrow). These morphological features are compatible with *L. blattarum* (immersion oil, $\times 1200$; bar, 15 µm). (b) Similar flagellate protozoon in a sputum smear, also demonstrating the piriform shape, granular cytoplasm and the tuft of flagella (black arrow) (Wheatley's trichomic, $\times 1000$; bar, 15 µm).

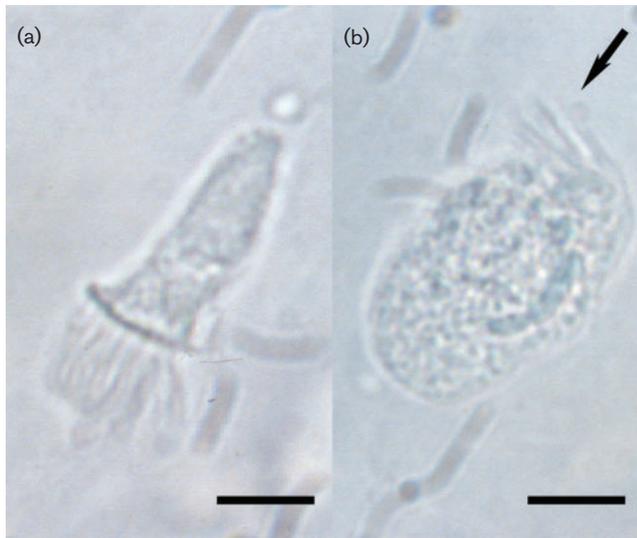


Fig. 2. Morphological differences between a ciliated bronchial cell and the flagellate protozoon *L. blattarum* in fresh sputum samples. (a) Ciliated bronchial cell with columnar shape, nucleus at basal end, visible and marked terminal bar and inserted cilia at the apical end. (b) *L. blattarum* with oval shape, granular cytoplasm with phagocytosed particles and a tuft of flagella (black arrow) (immersion oil $\times 1000$; bar, 6 μm).

such as the calyx, axial filament and parabasal body (Beams & Sekhon, 1969). The calyx is a trumpet-shaped body extending down the central axis, inside which is a nucleus. Surrounding the calyx is a specialized collar of cytoplasm known as the parabasal body, which possess numerous radiating tubules that penetrate to the calyx and supporting the nucleus–endomembrane system in a restricted region of the cell. This parabasal body is an endomembrane system restricted in position to a perinuclear zone at the anterior end of the protozoon. This endomembrane system may be involved in the synthesis of enzymes, lysosomes and peroxisomes (Kessel & Beams, 1990). In addition, the flagella–nucleus–parabasal body–calyx–axial filament complex may separate from the remainder of the cell and assume a temporary motile existence.

Although cilia and flagella have similar structures they can be differentiated as they vary in length, in number per cell, and in the patterns of motility that they produce (Mitchell, 2007; Lindeman & Lesich, 2010). However, in the opinion of the authors, misidentification of ciliated epithelial cells as flagellated protozoa may be present in a number of images in the published literature (Wang *et al.*, 2006; Yao *et al.*, 2009; He *et al.*, 2011).

Culture

L. blattarum is more difficult to culture than many other protozoa living in the gut of cockroaches such as *Endamoeba blattae* and *Nyctotherus ovalis*. However, it has been grown in three mediums utilized by Chen (1933)

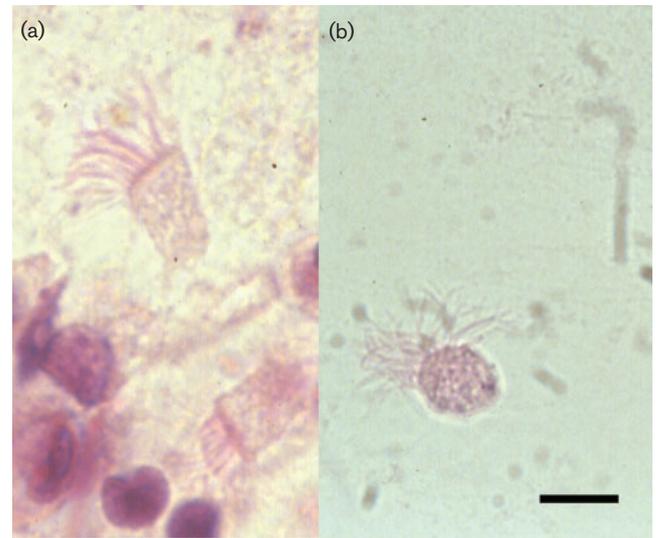


Fig. 3. Morphological differences between cytoplasmic fragments of ciliated bronchial cells (a) and the flagellate protozoon *L. blattarum* (b) in stained sputum smears. Note that cilia are regularly oriented whereas the flagella have an irregular arrangement (Papanicolaou stain, $\times 1000$; bar, 12 μm).

and in a medium which contained 0.8 % salt solution with yeast added as food (Kirby, 1950). The yeast used as the food source was grown in lemon juice diluted with an equal amount of tap water.

Epidemiology

Overt respiratory infection by *L. blattarum* is rare. We have identified 61 published case reports, of which 53 (86.9 %) were from China, six (9.8 %) from Perú and two (3.3 %) from Spain. Of the 53 Chinese cases, 37 were male and 16 female. Overall, 70.9 % (39/55) of cases where gender was recorded were male.

Most of the cases from outside Peru were in adults. The Chinese cases ranged from 15 to 95 years (median 45 years), the two Spanish cases were 21 and 45 years, respectively, and the Peruvian cases included two infants of 4 months and one of 18 months, and children aged 5, 8 and 15 years.

More cases were male than female: of the 45 Chinese cases, 31 were male and 14 females; the two Spanish cases were also male. The gender of the Peruvian cases was not recorded. Overall, 70 % (33/47) of cases where gender was recorded were male. The reasons for this gender imbalance are unclear.

All the cases in the reviewed literature had evidence of previous and/or concomitant respiratory disease. Immunosuppression was a feature in a number of the case reports, with seven cases having a history of renal allograft transplantation, one with liver allograft transplantation, and one case where allogeneic haematopoietic stem cell

Table 1. Cases reported on *L. blattarum* and bronchopulmonary disease

Author (s) and Year	Case (s) reported	Country
Chen & Meng (1993)	1	China
Dong <i>et al.</i> (2000)	1	China
He <i>et al.</i> (2011)	2	China
Kang <i>et al.</i> (2008)	1	China
Li <i>et al.</i> (2009)	1	China
Liu <i>et al.</i> (2007)	1	China
Martínez-Girón <i>et al.</i> (2007)	1	Spain
Martínez-Girón & Doganci (2010)	1	Spain
Miao <i>et al.</i> (2008)	1	China
Shi <i>et al.</i> (2007)	26	China
Wang (2012)	1	China
Wang <i>et al.</i> (2006)	4	China
Yang <i>et al.</i> (2000)	1	China
Yao (2008)	2	China
Yao <i>et al.</i> (2009)	1	China
Yao <i>et al.</i> (2008)	1	China
Yao <i>et al.</i> (2008)	1	China
Yao <i>et al.</i> (2008)	1	China
Yao <i>et al.</i> (1999)	1	China
Zerpa <i>et al.</i> (2010)	6	Peru
Zhang, F. <i>et al.</i> (2010)	2	China
Zhang, R. S. <i>et al.</i> (2010)	1	China
Zhang <i>et al.</i> (2011)	1	China
Zhou <i>et al.</i> (2006)	2	China
Total	61	

transplantation had taken place. Two of the Peruvian cases suffered from congenital heart disease and one from Down's syndrome, which may also have affected underlying immunocompetence. No case reports were identified in relation to human immunodeficiency virus infection, although this is an important cause of immune-suppression.

Clinical symptoms, diagnosis and treatment

In adult patients, the commonest clinical symptoms were similar to other respiratory conditions such as bronchial asthma, pneumonia, bronchiectasis or pulmonary abscesses. In the case reports, cough was always present. Varying degrees of expectoration were reported including small quantities of white sputum, yellowish purulent sputum or blood-stained sputum. Fever was present in over 90% of the English language cases where this question was addressed, with temperatures ranging from 37.5 to 39.0 °C. Some patients had shortness of breath, chest tightness, symptoms of acute wheeze and asthma attacks. On physical examination, many cases had fine crepitations and wheezing in both lungs.

Chest X-ray and CT scans showed patchy nodular or linear infiltrating opacities scattered throughout both lungs, which may be migratory, and associated with a degree of bronchial obstruction. Bronchoscopy has indicated affected airways that were narrowed with obstruction of the

bronchial orifices and a bronchial mucosa that appeared congested and oedematous, with foci of hyperplasia and inflammation and white necrotic matter on the mucosa. Examination of blood samples indicated that around 35% of patients had raised circulating eosinophils.

Diagnosis was generally made on the basis of observation of the flagellate protozoa in either fresh or stained sputum smears. In the case reports, respiratory secretions were obtained by means of bronchoscopy and/or spontaneous expectoration. Some of the patients were severely affected. Among the paediatric patients, five had pneumonia, one had atelectasis, and four were admitted to intensive care. There is a need for further research which applies modern molecular techniques to the characterization and diagnosis of these protozoa.

Treatment of bronchopulmonary infection by *L. blattarum* was generally with metronidazole, as this is commonly used in protozoal infection (Ribas *et al.*, 2007; Shi *et al.*, 2007). The usual dose is 500 mg every 8 h orally for 7–10 days in adults, and 7.5 mg kg⁻¹ every 8 h in children. A single intravenous dose of 15 mg kg⁻¹ over 1 h (as a loading dose) followed by 7.5 mg kg⁻¹ every 6 h has also been used. Metronidazole diffuses well throughout the body and is present in secretions and effusions. Excretion is by the renal route and consequently the dosage should be adjusted in the presence of renal insufficiency. The drug is not advised for use during pregnancy and lactation; it also interacts with alcohol to produce an adverse reaction. Alternatives would be Tinidazole 500 mg every 12 h orally for 5–6 days, or Albendazole 400 mg day⁻¹ orally for 5 days.

Conclusions

L. blattarum is increasingly being recognized as an important pulmonary protozoal infection (Vijayan & Kilani, 2010). This may in part be due to a rise in the number of individuals who have compromised immune function and are consequently susceptible to atypical infections (Ribas *et al.*, 2005; Duboucher *et al.*, 2005), including infection with species of protozoa that are not parasitic under normal circumstances (Barratt *et al.*, 2010).

Although immunosuppression was a contributory factor in a number of cases, it was not present in the majority of case reports identified for this review. In contrast, respiratory pathology was generally present either as a concomitant condition or subsequent to the infection. Diagnosis has generally been by light microscopy. However, the use of features identified under electron microscopy may be worth pursuing, as its use as a diagnostic tool has been demonstrated in relation to the presence of other flagellated protozoa in the respiratory tract (Carter & Whithaus, 2008).

Molecular studies have been undertaken to establish the phylogenetic position and taxonomic classification and of a wide range of endocommensal parabasalids (Gerbod *et al.*, 2000, 2004; Hampl *et al.*, 2004; Ohkuma *et al.*, 2005;

Yubuki *et al.*, 2010; Malik *et al.*, 2011). Similar studies have examined *L. striata* (Gile & Slamovits, 2012) and subsequently described a new genus (*Simplicimonas*) and three new species including *Tetratrichomonas undula*, *Hexamastix coercens* and *Simplicimonas similis* (Cepicka *et al.*, 2010). Some studies of human respiratory secretions and pleural fluid samples for flagellates have been undertaken, including studies of *Trichomonas vaginalis* (Duboucher *et al.*, 2003), *Trichomonas tenax* (Mahmoud & Rahman, 2004; Mallat *et al.*, 2004; Leterrier *et al.*, 2012), *Tetratrichomonas* sp. (Kutisova *et al.*, 2005; Mantini *et al.*, 2009) and *Tritrichomonas foetus* (Duboucher *et al.*, 2006). However, no molecular study of *L. blattarum* was identified.

Possible links between *Lophomonas* infection and insects in the domestic environment

Lophomonas may be an example of interplay between endosymbionts in insects found in domestic environments and human disease which may be occurring with other organisms. Several papers have proposed that cockroaches act as carriers of important carriers of human pathogens including protozoa, such as *Entamoeba histolytica* (Pai *et al.*, 2003; Kinfu & Erko, 2008), *Toxoplasma gondii* (Chinchilla *et al.*, 1994), *Cryptosporidium parvum* and *Balantidium coli* (El-Sherbini & Gneidy, 2012). The fact that *L. blattarum* is also carried by such insects suggests that the presence of infected insects in domestic dwellings could be associated with an increased risk of inhalation of *Lophomonas* and subsequent human infection. This hypothesis is supported by a number of considerations. It is presumed that *L. blattarum* present in the gut of cockroaches is subsequently eliminated from the hindgut in faeces and, as has been shown for another flagellate protozoa (Bittencourt-Silvestre *et al.*, 2010; Zaragatzki *et al.*, 2010), that the organism develops cystic forms that can survive in the environment if external conditions are adverse. It seems reasonable to suppose that cysts of *L. blattarum* could subsequently be inhaled into the respiratory airways, and given suitable humidity, temperature, and oxygen concentration, could undergo excystation. This would produce free trophozoites in the respiratory airway epithelium and it is possible to speculate that this could affect the human host via protease-activated receptors (Martínez-Girón & Ribas, 2006) and/or interaction with 'tight junctions' (Martínez-Girón, 2011a).

L. blattarum is an endocommensal in a range of insects found in the domestic environment. Cockroaches are known to harbour a considerable range of endocommensal ciliates, amoebae and flagellates including *L. blattarum*, *L. striata*, *Trichomastix orthoptera* and *Hexamita periplanetae* (Hegner, 1929) and constitute an important reservoir for infectious pathogenic parasites (Tatfeng *et al.*, 2005). On occasions cockroaches have been shown to feed on human faeces and subsequently disseminate cysts of enteric protozoans in the human faeces into the wider environment (Graczyk *et al.*, 2005).

There are a number of factors that support the hypothesis that the inhalation of *Lophomonas* cysts may be a route of transmission of this protozoon from cockroaches or other insects to humans. Protozoan parasites can encase themselves in carbohydrate-rich cysts which can survive under adverse conditions in soils and airborne environments before transmission to a new host (Eichinger, 2001; Chávez-Munguía *et al.*, 2007) and the encystation pathway is a recognized virulence mechanism whose 'biological goal' is survival in the environment and subsequent infection of a new host (Lauwaet *et al.*, 2007).

Many of the steps in this pathway have been demonstrated for *Lophomonas*. Protozoa have been shown to be living in the anaerobic environment as the gut of termites and cockroaches (Leschine, 1995). Cystic forms of flagellate protozoa (*Leptomonas* sp.) have been isolated from the intestine of insects which form the natural host of that species (Romeiro *et al.*, 2000). Cyst forms and excystation phenomena have also been observed in the hindgut of cockroaches in relation to *L. blattarum* and *L. striata*. Protozoan cysts of flagellates have been isolated as airborne contaminants in the atmosphere (Rogerson & Detwiler, 1999). In conclusion, although not proven, this hypothesis clearly warrants further examination. A similar hypothesis, that protozoa observed in sputum may be linked to endocommensal organisms in other respiratory conditions, has been proposed in relation to asthma (Martínez-Girón *et al.*, 2008b; Martínez-Girón, 2011b; Martínez-Girón & van Woerden, 2013; van Woerden *et al.*, 2011).

A clinical response to metronidazole would support a diagnosis of bronchopulmonary lophomoniasis where protozoa were identified in sputum. However, such a clinical response would not be pathognomonic, as metronidazole is also effective against a wide range of anaerobic bacteria and other parasitic respiratory infections (Samuelson, 1999). Interestingly, metronidazole has also been shown to have a major effect on intestinal protozoa present in the cockroach *P. americana* (Bracke *et al.*, 1978).

This review highlights some important areas for future work. Electron microscopy studies of *L. blattarum* are needed which might be of use in a diagnostic context. The development of specific culture media for the organism and the development of reliable molecular markers would also be very helpful. These steps would facilitate fuller characterization of this potentially important organism and clarification of the true prevalence of lophomoniasis.

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