

The prevalence of protozoa in the gut of German cockroaches (*Blattella germanica*) with special reference to *Lophomonas blattarum*

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Abstract The German cockroach (*Blattella germanica*) is a common domestic pest, which produces allergens that have been associated with broncho-pulmonary disease. Various protozoan species have been identified in the intestine of this cockroach and it has been hypothesised that these protozoa, or their proteases, may contribute to the burden of cockroach-associated allergens and adjuvants present in domestic dust. The aim of this study was therefore to determine the prevalence of protozoan species in the intestine of *Blattella germanica*. German cockroaches were anaesthetised and dissected and gut contents are used to produce wet slides for microscopy. Both, Giemsa and Papanicolaou stains were used to confirm correct identification of *Lophomonas blattarum*. Representatives of four genera of protozoa were identified in 110 cockroaches: *Nyctoterus* sp. was observed in 91.8% of cases, *Gregarina* sp. in 64.5%, *Amoeba* sp. in 25.4% and *Lophomonas blattarum* in 13.6%. *Nyctoterus* and *Gregarina* were statistically significantly more likely to be found in diseased cockroaches compared to *Amoeba* or *Lophomonas*. The prevalence of *Lophomonas blattarum* was similar to that in published studies of a different species of cockroach,

Periplaneta americana. Further work is needed to assess the interplay between protozoa, cockroaches and broncho-pulmonary diseases.

Keywords Protozoa · Cockroach · *Blattella germanica* · *Lophomonas blattarum*

Introduction

Cockroaches are among the most common of insects and are one of the most successful life forms. Because cockroaches are so adaptable, they have successfully adjusted to living in a wide range of environments and often co-exist with human populations. Although around 4500 species of cockroaches exist worldwide, 30 species are particularly adapted to human habitats and are recognised as domestic pests. Among the most common species of pest are *Blattella germanica* (German cockroach), *Periplaneta americana* (American cockroach), *Blatta orientalis* (Oriental cockroach), and *Supella longipalpa* (Brown-banded cockroach) (Nasirian 2017).

Blattella germanica, which is the focus of this paper, is strictly synanthropic, inhabiting human habitats, where they are close to food and moisture sources. This cockroach has a worldwide distribution. Adult German cockroaches are 13–16 mm long and light tan to brown in colour. They have fully developed wings, but do not fly. Nymphs are similar in appearance to adults but they are smaller and lack wings. The German cockroach is most easily identified by its small size and by two dark parallel lines running from the back of the head to the wings.

The presence of cockroaches in the domestic environment is an important public health issue. They are a common source of allergens and a major cause of asthma morbidity, especially

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in children and in inner-city homes (Rabito et al. 2011). They can also mechanically transmit pathogenic microorganisms including antibiotic-resistant strains of bacteria (Pai et al. 2005; Elgderi et al. 2006), parasites (helminths and protozoa), and fungi (Tatfeng et al. 2005). In that regard, *Blattella germanica* is of special interest as it has been considered a carrier and potential vector for medically important microorganisms in the hospital environment (Fotedar et al. 1991; Salehzadeh et al. 2007; Fakoorziba et al. 2010; Oliva et al. 2010; Menasria et al. 2014).

The presence of protozoa in the gut of the cockroaches has been recognised for a number of years (Armer 1944; De Coursey and Otto 1956; Tsai and Cahill 1970). The relationship is an ancient one, as Cretaceous protist flagellates (phylum *Parabasalia* and *Oxymonada*) have even been found in fossil cockroaches identified in pieces of Burmese amber (Poinar 2009).

Some of the intestinal protozoa in cockroaches are symbionts providing a substantial contribution to metabolic activity in the hindgut, as in the case of the ciliated protozoon *Nyctotherus ovalis* (Gijzen and Barugahare 1992). Other intestinal protozoa such as *Gregarina* may cause infection in cockroaches, producing swollen abdomens and slower movement in infected specimens. In some dead cockroaches, the infection presents as darkened bodies with a putrid smell, which may be the result of protozoal septicaemia (Lopes and Alves 2005; Yahaya et al. 2017). Clopton and Gold (1996) reported on the host specificity of *Gregarina blattarum* across five species of domiciliary cockroach: *Blattella germanica*, *Supella longipalpa*, *Blatta orientalis*, *Periplaneta americana*, and *Periplaneta fuliginosa*. *G. blattarum* infections were observed in all experimentally infected *B. germanica*, whereas no infections were observed in the rest of the studied species.

This paper has a particular focus on *Lophomonas blattarum*. The organisms were first described by Stein in 1860 as a multi-flagellated protozoon identified in the intestinal canal of the cockroach *Blatta orientalis* (Schuster 1898). The protozoon was later observed by Kudo in the same species of cockroach (Kudo 1926). Since then, the presence of this protozoon in the intestine of cockroaches has been reported in a number of papers and its morphological features have been characterised (Hoyte 1961; Beams and Sekhon 1969; Strand and Brooks 1977; Kessel and Beams 1990; Alam-Eldin and Abdulaziz 2015). However, the relative prevalence of different protozoa in the gut of cockroaches has had limited investigation.

The prevalence of different protozoa in the gut of cockroaches is potentially relevant to research into human respiratory disease. *Lophomonas blattarum* has been observed in the respiratory tract of human subjects and may be related to broncho-pulmonary disease (Martínez-Girón and van Woerden 2013; Martínez-Girón and van Woerden 2014a, b). We have previously hypothesised that

protozoa in the gut of cockroaches are likely to be excreted into faecal pellets and constitute part of the dust inhaled by human subjects inhabiting the same environment (Martínez-Girón and Ribas 2006).

The aim of this study was therefore to assess the prevalence of different protozoa in the gut of the German cockroach *Blattella germanica*, as this is a common pest in the domestic environment.

Materials and methods

Cockroaches were caught by means of plastic jar traps, which were left overnight around the kitchens of the old Central University Hospital of Asturias, Spain, during the summer months. This area has a temperate but humid climate that suits cockroaches. At the laboratory, each of the cockroaches was anaesthetised inside a glass bell jar using cotton soaked in ether and classified, based on developmental stage and gender, before being dissected to remove the intestinal tract. The dissection was performed using special scissors under a stereo microscope by cutting the ventral aspect of the abdomen along its length to remove the exoskeleton. The complete alimentary canal was removed (Fig. 1) and sectioned and the foregut, midgut and hindgut were cut longitudinally to expose the internal surface. Each section was then smeared onto a glass slide. A drop of normal saline was added and each wet smear was examined under a light microscope for the presence of protozoa. Where *Lophomonas blattarum* was observed, the smears were stained by Giemsa stain and the Papanicolaou method to confirm correct identification.

Results

A total of 110 adult German cockroaches were trapped (65 males and 45 females). Four protozoa were observed under light microscopy of wet smears: *Nyctotherus* sp. (Fig. 2), *Gregarina* sp. (Fig. 3), *Amoeba* sp. (Fig. 4), and *Lophomonas blattarum* (Fig. 5). A Giemsa stain of *Lophomonas blattarum* is provided in Fig. 6. The following morphological characteristics were assessed in both fresh and stained smears to identify *Lophomonas blattarum*: pyriform, oval or round shape; size of 20–60 µm; granular cytoplasm containing numerous vacuoles; a tuft of long, undulating flagella at one end of the cytoplasm with flagella that were longer at the centre of the tuft compared to the flagella at the edge of the tuft; a nucleus (where visible) that was located close to the site where the tuft of flagella was inserted into the cell wall. For *Nyctotherus* sp., the morphological characteristics used in identification were ovoid cell body (145–200 × 90–130 µm), a transversal macro-nucleus located in the anterior third of the cell above the equatorial part of the cell and with rows of cilia



Fig. 1 Entire alimentary canal removed from a cockroach (*Blattella germanica*) after its dissection

covering the entire surface. For *Gregarina* sp., the morphological characteristics used in identification were as follows: a cylindrical shape, ranging from 100 to 2000 μm in length and from 30 to 1500 μm in width, with bodies that were divided into two segments by a transverse partition, with the nucleus located in the larger segment. For *Amoeba* sp., the morphological characteristics used in identification were as follows: an irregular cytoplasm due to the presence of pseudopods, with a large nucleus, which was round in shape.

Table 1 summarises the frequent with which protozoa were observed. *Nyctotherus* sp. was observed in 91.8% of samples and *Lophomonas blattarum* in 13.6%. *Nyctotherus* sp. was observed in all three gut sections, whilst other protozoa species were only observed in the midgut and the hindgut of the cockroaches.

The prevalence of different protozoal species was compared between cockroaches that were well and those that were diseased using Fisher's exact test (Table 2). *Gregarina* sp. were more likely to be observed in diseased cockroaches than *Amoeba* sp. ($p = 0.026$) or *Lophomonas* sp. ($p = 0.033$). Similarly, *Nyctotherus* sp. was more likely to be observed in diseased cockroaches than *amoeba* sp. ($p = 0.0005$) or

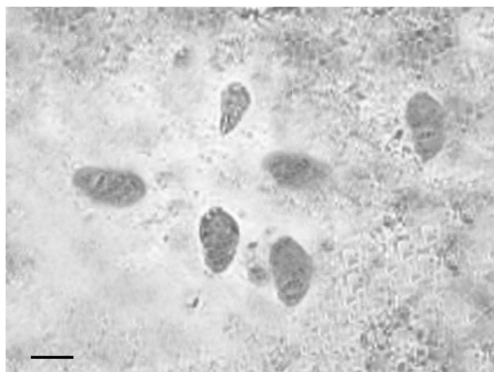


Fig. 2 *Nyctotherus* observed in a fresh wet smear from a hindgut of the German cockroach ($\times 400$, scale bar = 100 μm)

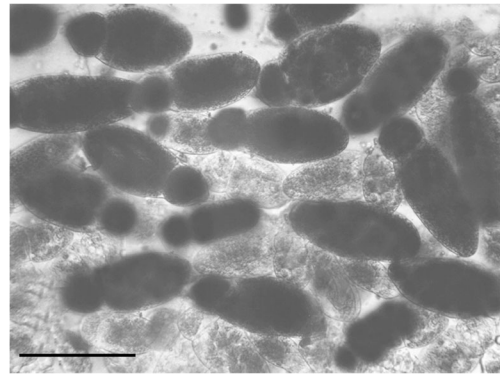


Fig. 3 *Gregarina* observed in a fresh wet smear from a hindgut of the German cockroach ($\times 400$, scale bar = 500 μm)

Lophomonas sp. ($p = 0.020$). *Gregarina* sp. was associated with the presence of putrid fluids when the cockroaches were dissected and the number of *Gregarina* sp. was greater in the intestines of diseased cockroaches and protozoa were observed in the cockroach egg cases (oothecas) of diseased or dead cockroaches.

Discussion

In this study, the prevalence of protozoa (*Nyctotherus* sp., *Gregarina* sp., *Amoeba* sp. and *Lophomonas blattarum*) was observed in fresh wet smears under light microscope from the intestine of the German cockroach (*Blattella germanica*). *Nyctotherus* sp. was observed in 91.8% of cases, *Gregarina* sp. in 64.5%, *Amoeba* sp. in 25.4% and *Lophomonas blattarum* in 13.6%.

Domestic cockroaches are potential carriers and vectors for protozoa that are known human pathogens such as *Entamoeba histolytica*, *Toxoplasma gondii*, *Balantidium coli*, *Cryptosporidium parvum*, *Isospora belli*, *Giardia duodenalis* and *Sarcocystis muris* have all been recovered from the external/internal body or faeces of these arthropods, mainly in the form of cysts (Smith and Frenkel 1978; Chinchilla et al. 1994; Pai et al. 2003; Graczyk et al. 2005; Chamavit et al.

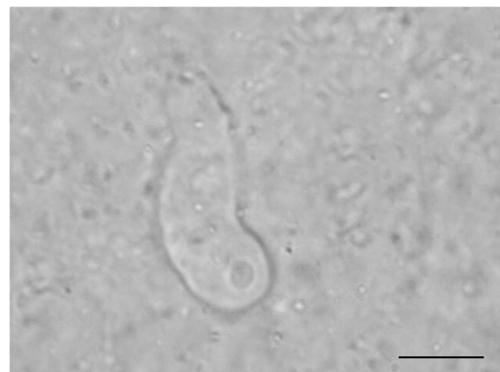


Fig. 4 *Amoeba* observed in a fresh wet smear from a midgut of the German cockroach (oil immersion $\times 1000$, scale bar = 50 μm)

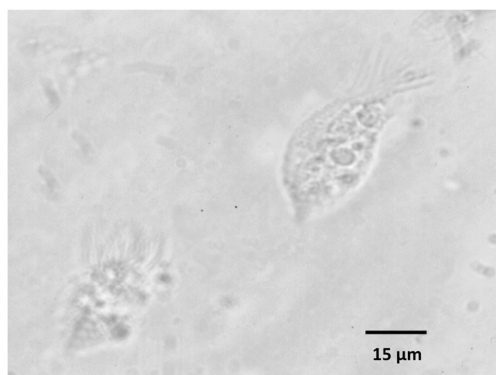


Fig. 5 Two trophozoites of *Lophomonas blattarum* observed in a fresh wet smear from a hindgut of the German cockroach (oil immersion $\times 1000$, scale bar = 15 μm)

2011; El-Sherbini and Gneidy 2012; Isaac et al. 2014; Hamu et al. 2014). *Lophomonas blattarum* has been observed in human samples from the respiratory tract (Martínez-Girón and Doganci 2010; Zepa et al. 2010), although it is difficult to distinguish from bronchial ciliated cells (Li and Gao 2016) and there has been some debate over its identification in some studies (Martínez-Girón and van Woerden 2014a, b; Martínez-Girón and van Woerden 2015; van Woerden and Martínez-Girón 2017; Martínez-Girón and van Woerden 2017). Given the difficulty in culturing *Lophomonas blattarum* and the absence of molecular markers, the strict application of morphological criteria continues to be important in the identification of this protozoon.

Few studies have examined the prevalence of *Lophomonas blattarum* in samples obtained directly from the alimentary canal of cockroaches, and such studies have been undertaken in a different species of cockroach, *Periplaneta americana* (Martínez-Girón et al. 2007; Yang et al. 2014; Cazorla Perfetti et al. 2015; Rina et al. 2015). Yang et al. observed *Lophomonas blattarum* in 40.0% (44/110), whereas other studies found a much lower rate: Perfetti et al. a prevalence of 8% (12/149) and Rina et al. a prevalence of 12.24% (6/49). In our study of *Blattella germanica*, the prevalence was 13.6%

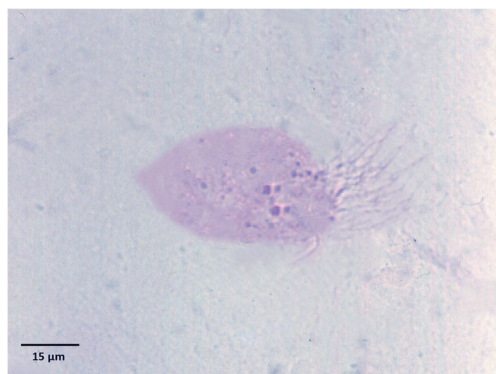


Fig. 6 Trophozoite of *Lophomonas blattarum* in a smear from a hindgut of the German cockroach. Note the pyriform-oval shape and the long and undulating tuft of flagella (Giemsa stain, $\times 1200$, scale bar = 15 μm)

Table 1 Protozoa observed in the guts of German cockroaches (GC), as well as its number in relation to the gender and section of the alimentary canal

Protozoa species	<i>Nyctotherus</i> sp.	<i>Gregarina</i> sp.	<i>Amoeba</i> sp.	<i>Lophomonas blattarum</i>
Males				
Foregut	11	0	0	0
Midgut	34	15	5	2
Hindgut	20	21	11	7
Females				
Foregut	6	0	0	0
Midgut	19	12	5	1
Hindgut	11	23	7	5
Total	101 (91.8%)	71 (64.5%)	28 (25.4%)	15 (13.6%)

(15/110). This is significantly lower than Tsai and Cahill (1970) who found *Lophomonas blattarum* in 47.6% of 105 cockroaches.

The study has a number of limitations. It could have been strengthened by samples of cockroaches drawn from a wider area, to assess any geographical or seasonal variation in the prevalence of protozoal species. The study would have been stronger if it had identified all of the parasitic taxa to a species level. There was some subjectivity in assessing whether or not cockroaches were diseased. Features that were applied included sluggish movement or the presence of purulent-like fluid in the cockroaches when dissected. However, these features are not definitive in assessing the health of these arthropods.

In addition to mechanical transmission of pathogenic protozoa, the presence of *Blattella germanica* in the environment is a recognised risk factor for asthma (Sohn and Kim 2012; Do et al. 2016). We have previously hypothesised a relationship between the flagellated protozoa observed in the intestinal extracts of cockroaches and the development of asthma (Martínez-Girón and Ribas 2006). This hypothesis is consistent with the fact that proteolytic enzymes, particularly cysteine proteases, have been described in several flagellated protozoan parasites (McKerrow et al. 1993). Allergen-derived proteases from the German cockroach have been suggested

Table 2 Prevalence of protozoa in well and diseased cockroaches

	Well cockroaches	Diseased cockroaches	Total
<i>Gregarina</i> species	53	18	71
<i>Nyctotherus</i> species	73	28	101
<i>Amoeba</i> species	28	0	28
<i>Lophomonas blattarum</i>	15	0	15
Total	169	46	215

as an adjuvant in the pathogenesis of allergic airway disease in mice models (Page et al. 2010; Day et al. 2012).

Further work is needed to assess the interplay between protozoa, cockroaches and broncho-pulmonary diseases. The prevalence of the vector and the parasite could be related to the prevalence of the disease in further future research to affirm the pathogenic role of *Lophomonas*. The development of reliable molecular markers for intestinal eukaryotes is also key to further research in this topic.

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