



Diagnosis of Pulmonary Lophomoniasis in Bronchoalveolar Lavage Samples from Patients with Persistent Cough and Different Diagnoses

Alba-Romero Jose de Jesus^{1*}, Garcia-Avila Luis-Uriel³, Alba-Romero Damari L² and Castro-Escarpulli Graciela³

¹Division of Postgraduate Studies and Research, Faculty of Chemical Sciences, Gomez Palace Unit, Juárez University of the State of Durango, Gomez Palace, Durango, Mexico

²Laboratory of Clinical Analysis Specialized Advisors of La Laguna, Gomez Palace, Durango, Mexico

³Clinical and Environmental Research Laboratory, Department of Microbiology, National School of Biological Sciences, National Polytechnic Institute, Mexico

***Corresponding Author:** Alba-Romero Jose de Jesus, Division of Postgraduate Studies and Research, Faculty of Chemical Sciences, Gomez Palace Unit, Juárez University of the State of Durango, Gomez Palace, Durango, Mexico.

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Alba-Romero Jose de Jesus., et al.

Abstract

Pulmonary lophomoniasis is a disease caused by *Lophomonas blattarum*, a multiflagellate protozoan of the parabasalid family. *L. blattarum* is a commensal that lives in the intestinal tract of cockroaches and helps them degrade cellulose and organic matter. It affects humans with diseases that leave them in a state of immunosuppression, mainly generating chronic productive cough, hemoptysis, fever, pleural effusion and, in severe cases, pneumonia and death. The main objective was to identify *L. blattarum* in bronchoalveolar lavage samples from patients with different diagnoses such as persistent cough, tuberculosis, bronchial asthma, bacterial infections, fungi and lung cancer. Fresh analysis of 75 bronchoalveolar lavage samples was performed in 2024, observing the centrifugation of the samples under a microscope. *L. blattarum* was found in 32 samples (42.7%), representing the incidence of blattarum cases in northern Mexico. It is concluded that pulmonary lophomoniasis is an emerging disease that requires training in microscopic identification and specific staining to establish the diagnosis and prescribe the correct treatment.

Keywords: Lophomonas; *Lophomonas blattarum*; Pulmonary Lophomoniasis

Introduction

Pulmonary lophomoniasis is a disease caused by multiflagellate protozoa of the Lophomonididae family of the genus *Lophomonas* and the species *blattarum* and *striata*; it is a commensal of the intestine of cockroaches [1]. Until a few decades ago it was a little-known disease, however, countries such as Iraq, China, Peru and Mexico have reported cases in patients of different ages [2]. The clinical picture in most patients is persistent and productive cough, fever, hemoptysis, in severe cases pleural effusion and

pneumonia. It is characterized by leukocytosis, neutrophilia and eosinophilia [3]. The diagnosis of lophomoniasis is mainly made by studying a fresh bronchoalveolar lavage (BAL) sample, observing the moving multiflagellate pear-shaped forms under a microscope; Microscopic observation is a useful but questionable tool because in many cases erroneous diagnoses have been made by confusing *Lophomonas* protozoa with goblet cells of the ciliated pseudostratified columnar epithelium [4]. However, the experience in the observation of *L. blattarum* trophozoites in fresh study and cor-

roborating with stains such as Gram stain and Ziehl Neelsen allows the diagnosis to have greater sensitivity. Another method used in recent years is the end-point polymerase chain reaction (PCR), this molecular method has evolved since the sequence currently used consists of 214 base pairs of a conserved sequence of the genus *Lophomonas*, this molecular method has a sensitivity of 99% and a specificity of 100% [5]. *L. blattarum* is a protozoan that affects humans when inhaled cysts, once in the lung they carry out the excystment process to pass to the active form, *L. blattarum* has proteolytic enzymes that help it degrade the tissue at the site where it is located. When it enters the lung where a lung lesion already exists, it favors it because it more easily evades the immune system [6]. It mainly affects immunosuppressed patients since it is an opportunistic anaerobic parasite where transplant patients, with kidney failure, cancer and chronic degenerative diseases [7]. Coinfection of *L. blattarum* with tuberculosis (TB), mycosis, Coronavirus disease 2019, better known as COVID-19 (COVID-19) is common and it is necessary to make the correct diagnosis to prescribe the correct treatment consisting of metronidazole 500 mg every 8 h for 10 or 12 days in adult patients, most patients evolve favorably and in children the dose is 75 mg/kg of weight every 8 hours for 10-15 days [8].

Materials and Methods

75 bronchoalveolar lavage samples from patients with different diagnoses were collected : Persistent productive cough (24), Productive cough in cancer patients (3), Productive cough with probable TB/Aspergillosis (12), Persistent productive cough due to bronchial asthma (5), Persistent productive cough in transplant patients (4), Productive cough diagnosed with TB (14), Persistent cough in patients with diabetes (10), Persistent cough in patients with HIV (3) (Figure 1). Once the bronchoalveolar lavage sample was taken by the specialist physician, it was taken to the laboratory for analysis in search of a causative agent of the lung problem. The study was performed fresh under a microscope by placing 50 μL on a centrifuge slide at 1400 rpm or 55 g of bronchoalveolar lavage and covering it with a 22X22 coverslip. It was observed with a 10X objective and subsequently with a 40X objective. Moving multiflagellate pear-shaped forms were searched for, differentiating between goblet cells of the ciliated pseudostratified columnar epithelium and *L. blattarum*. BAL samples were plated on 5% Sheep Blood Agar, MacConkey Agar, Chocolate Agar, Biggy Agar, and Sabouraud Agar; they were incubated at 36°C for 18-24 h except for Biggy Agar, which was incubated at room temperature for 10 days. Samples were handled in a class II A biosafety cabinet; bacterial identification was performed using the VITEK® 2 Compact automated microbial identification system.

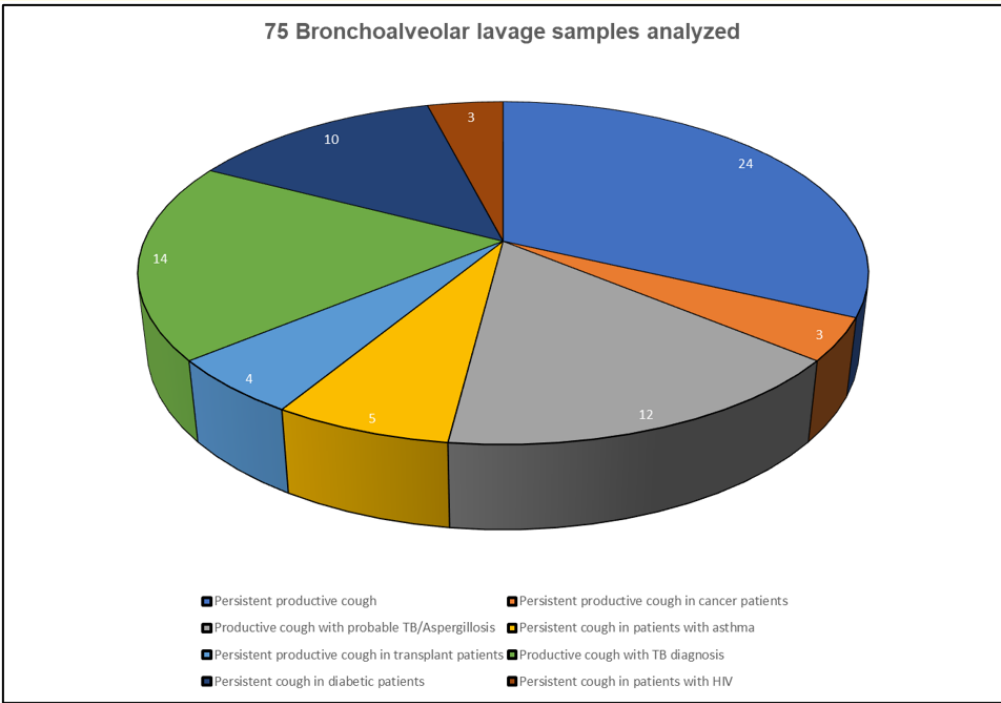


Figure 1: Classification of BAL samples and their underlying disease.

Results

Seventy-five BAL samples from patients with different diagnoses and underlying diseases were analyzed. Fresh samples were examined for *L. blattarum*. Thirty-two samples were positive for

L. blattarum using fresh samples under a microscope, Gram staining, and Ziehl-Neelsen staining (Figure 2). Eleven microorganisms were isolated in the bacteriological study, except for the acid-fast bacilli observed by direct bacilloscopy (Figure 3).

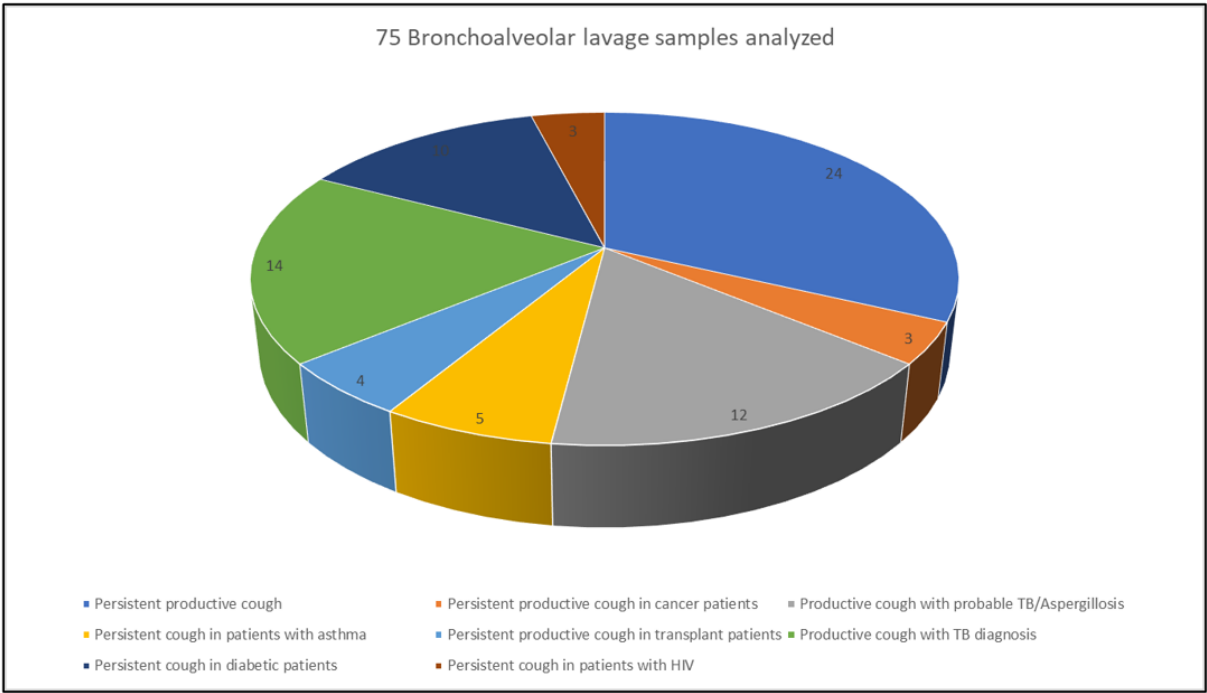


Figure 2: Positive sample results based on baseline samples.

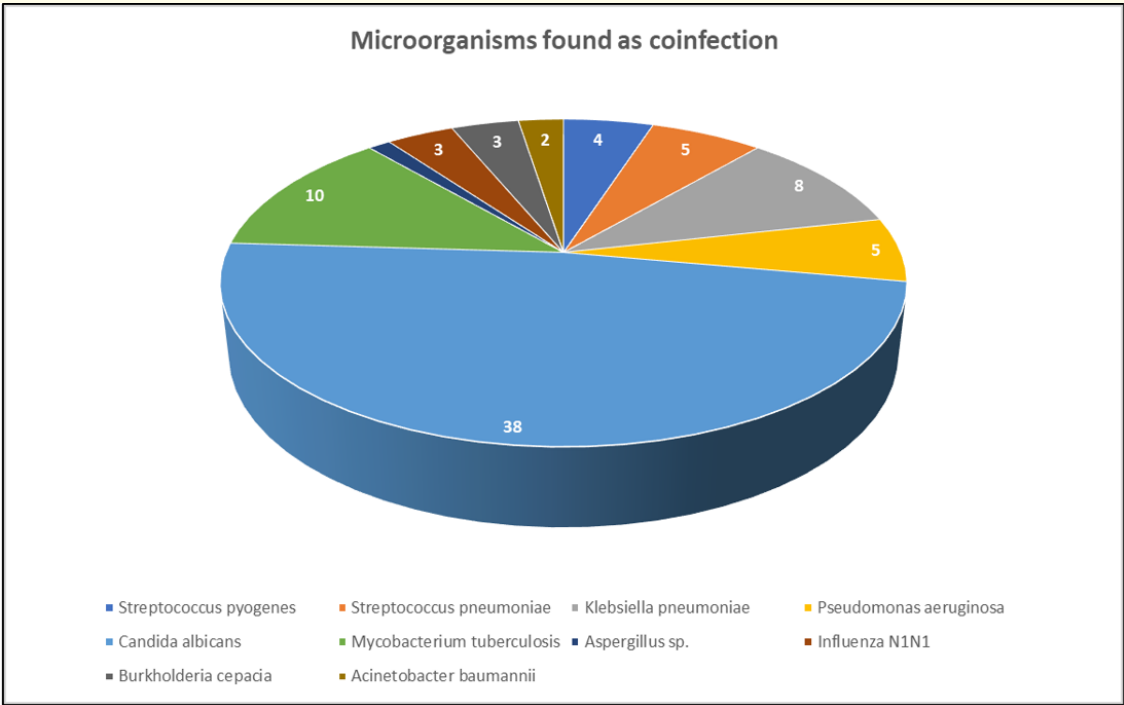


Figure 3: Microorganisms found in BAL samples as coinfection with *L. blattarum*.

Discussion

In this study, 75 BAL samples were analyzed from patients who had a persistent cough in common, some had a productive cough, and had been treated with multiple antibiotics. The characteristics of the patients for the diagnosis of pulmonary lophomoniasis coincide with those described by Kalani, Hamed., *et al.* (2022) and Veisi, Hossein., *et al.* (2024) when describing the most common symptoms of patients with pulmonary lophomoniasis, emphasizing chronic cough, sputum, dyspnea and sometimes hemoptysis [9], which is why it is necessary to make the diagnosis of TB [10]. The patients included in the study had in common the presence of chronic cough, however, each of the patients already had an underlying disease and a specific treatment without improvement, mainly cough. Prolonged treatments without favorable response is a characteristic that we find in patients, which coincides with that described by Jalayeri, Mohammad Hadi Tajik., *et al.* (2024) that prolonged treatments without improvement and treatments with immunosuppressants increase susceptibility [11]. By establishing the incidence of 42.7% with the fresh analysis method under the microscope, it is considered that it could be higher based on the sensitivity of the method and the experience of health personnel, therefore, we consider that a molecular method such as the dot PCR is a more efficient method as mentioned by Taheri, Amir-masoud., *et al.* (2024), they applied this method to make the diagnosis in BAL samples, they received correct treatment and the patients improved completely in 6 months [12]. Immunodeficiency in patients due to prolonged treatment or underlying disease is a common factor in patients and increases susceptibility, especially in patients who have been transplanted, as mentioned by Gheisari, Zahra., *et al.* (2020) in their study by stating that organ transplant recipients are considered immunosuppressed patients due to the prescription of immunosuppressive drugs. This group of patients is susceptible to opportunistic infections as well as lophomoniasis [13]. Diseases that decrease the patient's immunity such as autoimmune diseases are a risk factor for acquiring lophomoniasis, Wahid, Wathiqah., *et al.* (2019) found a case of bronchopulmonary lophomoniasis in a Malaysian woman with systemic lupus erythematosus. She presented with productive cough, dyspnea and high fever for two weeks [14]. Coinfection was an important factor found in patients with pulmonary lophomoniasis in this study, it is a factor that exacerbates the symptoms, it can be pathogenic bacteria, fungi and viruses as observed by Morán-Mariños, Cristian.,

et al. (2024) stating that lophomoniasis should be considered in patients with pneumonia who do not respond to antibiotic treatment, and epidemiological factors corresponding to polymicrobial coinfection, in addition to bronchoscopy, should be carefully considered for an accurate diagnosis [15].

Conclusion

The symptoms of a persistent, productive cough are exacerbated, especially when there is coinfection with other TB diseases, mycoses, or multidrug-resistant extended-spectrum beta-lactamase (ESBL) producing bacteria. However, persistent cough and the onset of fever are epidemiological findings associated with pulmonary lophomoniasis, an emerging disease that requires training in microscopic identification and specific staining to establish the diagnosis and prescribe the correct treatment.

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Author Contributions

The project design was led by José de Jesús Alba Romero, the collection of samples and preparation was led by Damari Lizeth Alba Romero, the evaluation of the samples for the fresh study under the microscope to observe *L. blattarum* was carried out by José de Jesús Alba Romero, bacteriological identification and quality control were performed by Graciela Castro Escarpulli.

Declaration of Conflicts of Interest

The authors of this manuscript declare that they have no conflicts of interest of any kind with their employer, institutions, or commercial companies.

Ethical Aspects

The patients signed the informed consent, as did their treating physicians.

The study was conducted according to the guidelines of the Declaration of Helsinki and approved under the guidelines of the Research Ethics Committee (CEI, folio 123301538X0201COFE-PRIS), with authorization for this work under registration R-2015—123301538X0201-017. In the Faculty of Chemical Sciences of the Juarez University of the State of Durango, Mexico, and the Federal Commission for the Protection against Sanitary Risks.

Informed Consent

The patients who participated in the research work signed a letter of informed consent voluntarily, declaring that they participated voluntarily and without any remuneration.

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