REVIEW

Protozoa in respiratory pathology: a review

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ABSTRACT: Among the micro-organisms that may affect the respiratory apparatus are the protozoa. The diseases they may give rise to constitute a relatively uncommon group of respiratory ailments with, in the majority of cases, an underlying clinical situation corresponding to states of suppressed immunity (AIDS, transplants, malign haemopathies, corticotherapy, *etc*.). Other factors, such as visits to endemic areas and immigration, also have to be taken into account.

In view of the probable increase in the number of cases and the appearance of new emerging diseases, it is the intention of the present work to review the publications available, in different fields of medicine, that refer to the principal kinds of protozoa (Entamoeba, Acanthamoeba, Balamuthia, Leishmania, Trypanosoma, Trichomonas, Lophomonas, Cryptosporidium, Cyclospora, Toxoplasma, Plasmodium, Babesia, Encephalitozoon, Enterocytozoon and Balantidium) and, at the same time, detail and comment on the latest findings on this subject.

KEYWORDS: Parasitic pulmonary diseases, protozoa, protozoal respiratory pathology

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STATEMENT OF INTEREST
None declared.

Protozoa are unicellular eukaryotic micro-organisms. Their morphology is varied, and their physiology and metabolism are adapted to their needs; nutrition is heterotrophic in the parasitic forms and autotrophic in the free-living ones, they have more or less complex life cycles, both free-living and parasitic, and in many cases, a vegetative form (trophozoite) and another resistant form (cyst).

Although at present there exists a tendency towards systematic unification on the basis of molecular phylogens of the ribosomic fraction of 18S [1], the established classification of the protozoa of greatest interest [2] consists of five phyla: Sarcomastigophora, Apicomplexa, Microspora, Mixozoa and Ciliophora.

The effects of protozoa on the respiratory apparatus, as opposed to other localisations (intestine, liver, vagina, urethra, *etc*.), constitute a group of rare diseases [3], with the existence, in most cases, of an underlying clinical situation corresponding to certain states of suppressed immunity (AIDS, transplants, malign haemopathies, corticotherapy *etc*.), although other factors, such as visits to endemic areas and immigration, also have to be taken into account [4].

The mechanisms by means of which the protozoa affect the respiratory apparatus may be: direct damage to the parenchyma (*e.g.* toxoplasmosis); through a systemic inflammatory response by haematogenous dissemination (*e.g.* malaria); and contiguity with an adjacent lesion (*e.g.* amoebiasis).

Although many of the protozoa that are of medical interest may be cultivated, this technique is little used for diagnostic purposes, enhancing the importance of microscopic visualisation. This may be achieved either by fresh examination or by the employment of specific stains (Wheatley's trichromic, Giemsa, Heidenhain, Lugol, *etc*.). Furthermore, together with the electron microscope, there exist more specific diagnostic methods based on immunological analysis or molecular biology tests.

It is the intention of the present work to review the publications available, in different fields of medicine, that refer to the principal kinds of protozoa that may affect the respiratory apparatus, presenting them in a single monographic paper, and at the same time detail and comment on the latest findings on this subject, one which may well, in the relatively near future, open up new fields of research in this area of respiratory pathology.

The literature review was identified through electronic databases, such as MEDLINE, EMBASE and the Cochrane Database of Systematic Reviews. The search was performed using the keywords ''protozoa'', ''lung'' and ''pulmonary infection'', and the names of each kind of protozoon implicated in the study.

Peer-reviewed publications in English, French and Spanish languages, along with English-language abstracts of non-English papers identified in the present research, were included. Inclusion or exclusion of each paper was decided mainly by considering the relevance of the article, journal impact factor, number of patients included in the work and pathological findings.

PATHOGENIC AGENTS AND CLINICAL SITUATIONS

Table 1 shows the different types of protozoa that may cause pathology in the respiratory apparatus. Preferred route and duration of therapy for each organism are summarised in table 2.

Phylum Sarcomastigophora

This phylum includes the subphylum Sarcodina (amoebae) and Mastigophora (flagellates).

The general characteristic of the Sarcodina subphylum is that it presents mobile trophozoites, inconstant in shape, in the form of pseudopods, although some may present temporary flagellae. However, in the Mastigophora subphylum, the trophozoites are constant in shape and have one or two flagellae, occasionally associated with other organelles, both at the base and as undulating membranes.

Genus Entamoeba

Within this genus, the only species traditionally recognised as a pathogen for humans, who are its principal host and reservoir, is *Entamoeba histolytica*. This protozoon is distributed worldwide, although it is more frequent in countries where health conditions are deficient. Malnutrition, advanced age, pregnancy, immune suppression states, alcoholism and certain sexual practices are risk factors favouring its development. In the present context, visits to endemic areas (Africa, Asia and Central America) and immigration are two additional factors to be taken into account.

This is the agent responsible for clinical situations such as traveller's diarrhoea, amoebic diarrhoea and amoebic dysentery, all of which are consequences of infection of the large intestine, as well as for extraintestinal amoebiases due to trophozoites (fig. 1) acceding to the blood vessels and then being carried in the bloodstream to various organs, such as the liver, lung, brain, heart, skin, *etc*.

Pleuropulmonary infection by *E. histolytica* is, after that of the liver, the most frequent form of extraintestinal amoebiasis and occurs, in most cases, as a complication of a hepatic abscess (fig. 2a and b) due to fistulisation [6–8], with the lower lobule of the right lung being the area most frequently affected. However, the haematogenous path has also been described as a cause of pulmonary lesions due to *E. histolytica* [9, 10].

The clinical situation largely depends on the associated pulmonary pathology, frequent symptoms being fever, cough, haemoptysis and pleuritic pain [11, 12]. On occasion, a hepatic abscess may provoke venous compression and be the cause of respiratory distress and alterations in arterial gasometry [13]. The appearance of a purulent fluid of a chocolate-like appearance (often described as anchovy paste) following the puncture of an abscess, discharge, or through vomiting, are highly suggestive signs [14].

Radiographic findings are variable, ranging from fine basal reticular patterns, with focal areas of atelectasis, to extensive infiltrates. Elevation of the right diaphragm (fig. 3), hepato-bronchial fistula, lung abscess, pleural effusion or discharge and bronchopleural fistula with pyopneumothorax are other lesions described [15–17].

Procedures such as sputum cytology or fine-needle puncture aspiration have proved their usefulness in the diagnosis of pulmonary lesions in which the diagnosis of pulmonary amoebiasis was not initially suspected [18, 19]. Moreover, in cases of pulmonary amoebiasis without data indicating intestinal and/or hepatic repercussions, which have to be distinguished from other processes, such as bacterial abscesses, neoplasias, empyema and tuberculosis [20], the combination of trophozoite visualisation through cytological sputum examination with DNA extraction techniques has proved to be of

TABLE 1	Protozoa involved in pathology of respiratory apparatus

Kingdom: Protista
  Subkingdom: Protozoa
    Phylum: Sarcomastigophora
      Subphylum: Sarcodina
        Superclass: Rhizopoda
          Order: Amoebida
            Family: Endamoebidae (Genus: Entamoeba)
          Order: Schyzopyrenida
            Family: Leptomyxidae (Genera: Acanthamoeba and Balamuthia)
      Subphylum: Mastigophora
        Order: Trichomonadida
          Family: Trichomonadidae (Genera: Trichomonas, Tritrichomonas and Tetratrichomonas)
        Order: Hypermastigida
          Family: Hypermastigidae (Genus: Lophomonas)
        Order: Kinetoplastida
          Family: Trypanosomatidae (Genera: Leishmania and Trypanosoma)
    Phylum: Apicomplexa
      Class: Sporozoea
        Subclass: Coccidia
          Order: Eucoccidiida
            Family: Cryptosporidiidae (Genus: Cryptosporidium)
            Family: Eimeriidae (Genus: Cyclospora)
            Family: Sarcocystidae
              Subfamily: Toxoplasmatinae (Genus: Toxoplasma)
            Family: Plasmodiidae (Genus: Plasmodium)
        Subclass: Piroplasmia
         Order: Piroplasmida
          Family: Babesiidae (Genus: Babesia)
    Phylum: Microspora
      Class: Microsporea
        Order: Microsporidia
          Family: Glugeidae (Genus: Encephalitozoon)
          Family: Pereziidae (Genus: Enterocytozoon)
    Phylum: Ciliophora
      Class: Kinetofragminophorea
        Order: Trichostomatida
          Family: Balantidiidae (Genus: Balantidium)

TABLE 2 The most relevant first-choice drug for protozoa in respiratory pathology

Parasite species and drug	Adult dosage	Paediatric dosage
Entamoeba histolytica		
Metronidazole	750 mg _p.o._ _t.i.d._ for 7–10 days	35–50 mg·kg^{-1}·day^{-1} _p.o._ in 3 doses for 7–10 days
Tinidazole	2 g _p.o._ _q.d._ for 5 days	⩾3 yrs: 50 mg·kg^{-1}·day^{-1} (maximum 2 g) _p.o._ in 1 dose for 3 days
Naegleria fowleri, Acanthamoeba, Balamuthia, Sappinia		
Amphotericin B	1.5 mg·kg^{-1}·day^{-1} _i.v._ in 2 doses for 3 days, then 1 mg·kg^{-1}·day^{-1} for 6 days plus 1.5 mg·day^{-1} intrathecally for 2 days, then 1 mg·day^{-1} every other day for 8 days	1.5 mg·kg^{-1}·days^{-1} _i.v._ in 2 doses for 3 days, then 1 mg·kg^{-1}·day^{-1} for 6 days plus 1.5 mg·day^{-1} intrathecally for 2 days, then 1 mg·day^{-1} every other day for 8 days
Trichomonas vaginalis, Pentatrichomonas hominis, Lophomonas		
Metronidazole	2 g _p.o._ once or 500 mg _b.i.d._ for 7 days	15 mg·kg^{-1}·day^{-1} _p.o._ in 3 doses for 7 days
Tinidazole	2 g _p.o._ once	50 mg·kg^{-1} once (maximum 2 g)
Leishmania spp.		
Liposomal amphotericin B	3 mg·kg^{-1}·day^{-1} _i.v._ on days 1–5, 14 and 21	3 mg·kg^{-1}·day^{-1} _i.v._ on days 1–5, 14 and 21
Sodium stibogluconate	20 mg Sb·kg^{-1}·day^{-1} _i.v._ or _i.m._ for 28 days	20 mg Sb·kg^{-1}·day^{-1} _i.v._ or _i.m._ for 28 days
Miltefosine	2.5 mg·kg^{-1}·day^{-1} _p.o._ (maximum 150 mg·day^{-1}) for 28 days	2.5 mg·kg^{-1}·day^{-1} _p.o._ (maximum 150 mg·day^{-1}) for 28 days
Trypanosoma (Schizotrypanum) cruzi		
Nifurtimox	8–10 mg·kg^{-1}·day^{-1} _p.o._ in 3–4 doses for 90–120 days	1–10 yrs: 15–20 mg·kg^{-1}·day^{-1} _p.o._ in 4 doses for 90–120 days 11–16 yrs: 12.5–15 mg·kg^{-1}·day^{-1} in 4 doses for 90–120 days
Benznidazole	5–7 mg·kg^{-1}·day^{-1} _p.o._ in 2 doses for 30–90 days	⩽12 yrs: 10 mg·kg^{-1}·day^{-1} _p.o._ in 2 doses for 30–90 days ⩾12 yrs: 5–7 mg·kg^{-1}·day^{-1} in 2 doses for 30–90 days
Cryptosporidium spp.		
Nitazoxanide	500 mg _p.o._ _b.i.d._ for 3 days	1–3 yrs: 100 mg _p.o._ _b.i.d._ for 3 days 4–11 yrs: 200 mg _p.o._ _b.i.d._ for 3 days >12 yrs: 500 mg _p.o._ every 12 h for 3 days
Cyclospora cayetanensis		
Trimethoprim–sulfamethoxazole	Trimethoprim 160 mg, sulfamethoxazole 800 mg (1 double-strength tablet) _p.o._ _b.i.d._ for 7–10 days	Trimethoprim 5 mg·kg^{-1}, sulfamethoxazole 25 mg·kg^{-1}·day^{-1} _p.o._ in 2 doses for 7–10 days
Toxoplasma gondii		
Pyrimethamine	25–100 mg·day^{-1} _p.o._ for 3–4 weeks	2 mg·kg^{-1}·day^{-1} _p.o._ for 2 days, then 1 mg·kg^{-1}·day^{-1} (maximum 25 mg·day^{-1}) for 4 weeks
Sulfadiazine	1–1.5 g _p.o._ _q.i.d._ for 3–4 weeks	100–200 mg·kg^{-1}·day^{-1} _p.o._ for 3–4 weeks
Plasmodium falciparum or _Plasmodium_ spp. acquired in areas of chloroquine-resistant _P. falciparum_		
Oral treatment of malaria		
Atovaquone/Proguanil	2 adult tablets _b.i.d._ or 4 adult tablets _q.d._ for 3 days	<5 kg: not indicated 5–8 kg: 2 paediatric tablets _q.d._ for 3 days 9–10 kg: 3 paediatric tablets _q.d._ for 3 days 11–20 kg: 1 adult tablet _q.d._ for 3 days 21–30 kg: 2 adult tablets _q.d._ for 3 days 31–40 kg: 3 adult tablets _q.d._ for 3 days >40 kg: 4 adult tablets _q.d._ for 3 days
Plasmodium vivax acquired in areas of chloroquine-resistant _P. vivax_		
Oral treatment of malaria		
Mefloquine	750 mg _p.o._ followed 12 h later by 500 mg	15 mg·kg^{-1} _p.o._ followed 12 h later by 10 mg·kg^{-1}
All Plasmodium species except chloroquine-resistant _P. falciparum_ and chloroquine-resistant _P. vivax_		
Oral treatment of malaria		
Chloroquine phosphate	1 g (600 mg base) _p.o._, then 500 mg (300 mg base) 6 h later, then 500 mg (300 mg base) at 24 and 48 h	10 mg base·kg^{-1} (600 mg base) _p.o._, then 5 mg base·kg^{-1} 6 h later, then 5 mg base·kg^{-1} at 24 and 48 h
All Plasmodium species (chloroquine-sensitive and -resistant)		
Parenteral treatment of malaria		
Quinidine gluconate	10 mg·kg^{-1} _i.v._ loading dose (maximum 600 mg) in normal saline over 1–2 h, followed by continuous infusion of 0.02 mg·kg^{-1}·min^{-1} until _p.o._ therapy can be started	10 mg·kg^{-1} _i.v._ loading dose (maximum 600 mg) in normal saline over 1–2 h, followed by continuous infusion of 0.02 mg·kg^{-1}·min^{-1} until _p.o._ therapy can be started
Babesia spp.		
Clyndamycin	1.2 g _b.i.d._ _i.v._ or 600 mg _t.i.d._ _p.o._ for 7–10 days	20–40 mg·kg^{-1}·day^{-1} _p.o._ in 3 doses for 7–10 days
Quinine	650 mg _p.o._ _t.i.d._ for 7–10 days	30 mg·kg^{-1}·day^{-1} _p.o._ in 3 doses for 7–10 days
Enterocytozoon bieuneusi, Encephalitozoon spp.		
Albendazole	400 mg _p.o._ _b.i.d._	
Balantidium coli		
Tetracycline	500 mg _p.o._ _q.i.d._ for 10 days	40 mg·kg^{-1}·day^{-1} (maximum 2 g) _p.o._ in 4 doses for 10 days

Data are taken from [5].

FIGURE 1. *Entamoeba histolytica*: two trophozoites in faecal iodine-stained wet mount. Scale bar=15 µm.

great utility [21]. It is important to note that, in sputum samples, a nonpathogenic species, *Entamoeba gingivalis*, may appear, which is present in the oral cavity [22] and may be confused with *E. histolytica* [23, 24]. Complementary laboratory tests, such as serological detection of antibodies, also help establish a definitive diagnosis.

Genera Acanthamoeba and Balamuthia

A series of protozoa ubiquitously present in nature (they may be isolated from earth, air and water) and which may be pathogenic for humans, whether their condition is normal or immunosuppressed, have been grouped under the denomination ''free-living amoebae'' [25]. Knowledge of these protozoa is important for two reasons: on the one hand, they cause pathologies in various organs (brain, cornea, skin, lung, *etc.*), while on the other, they may harbour and transmit pathogenic bacteria [26–29], some of which, such as *Legionella pneumophilia*

[30, 31], mycobacterias [32, 33], *Chlamydia pneumoniae* [34] and *Francisella tularensis* [35], have important repercussions on the respiratory system. This last factor may have clinical relevance, as a series of micro-organisms associated with amoebae are mentioned as agents causing nosocomial pneumonia [36].

Of the genera known at present, only four are associated with human pathology: Acanthamoeba, Balamuthia, Naegleria and Sappinia. Studies carried out on experimental animals have shown that certain species, such as *Naegleria fowleri*, *Acanthamoeba castellanii* and *Acanthamoeba polyphaga* may cause direct damage to the pulmonary parenchyma in the form of pneumonitis, with thickening of the alveolar walls, mononuclear inflammatory infiltrate and development of hyaline membranes in the alveolar spaces [37–40]. In the case of humans, only Acanthamoeba spp. and *Balamuthia mandrillaris* have been related with pulmonary pathology [41–43].

Pneumonitis is the most frequent lesion, with the presence of cystic forms and trophozoites in the alveolar spaces. Radiological examination reveals diffuse areas of alveolar consolidation. The identification of trophozoites in bronchoalveolar lavage samples and their subsequent cultivation help establish a diagnosis [44, 45]. In other types of samples, such as nasal exudates, the presence of amoebae (Naegleria spp. and Acanthamoeba spp.) has been described [46].

Genera Trichomonas, Tritrichomonas and Tetratrichomonas

The literature reviewed shows that the three species of Trichomonas that are pathogenic in humans (*Trichomonas vaginalis*, *T. tenax* and *T. hominis*) are implicated in infectious processes that affect the respiratory apparatus.

Pulmonary infection by *T. vaginalis* (fig. 4) may appear in newborn babies [47, 48], adults presenting suppressed immunity [49, 50] and in patients with acute respiratory distress syndrome [51, 52]. In the case of newborn babies there exists the antecedent of vaginal birth by mothers infected with the protozoon [53, 54]. Complications may be immediate, in the form of respiratory difficulty [55], or long term [56]. Detection
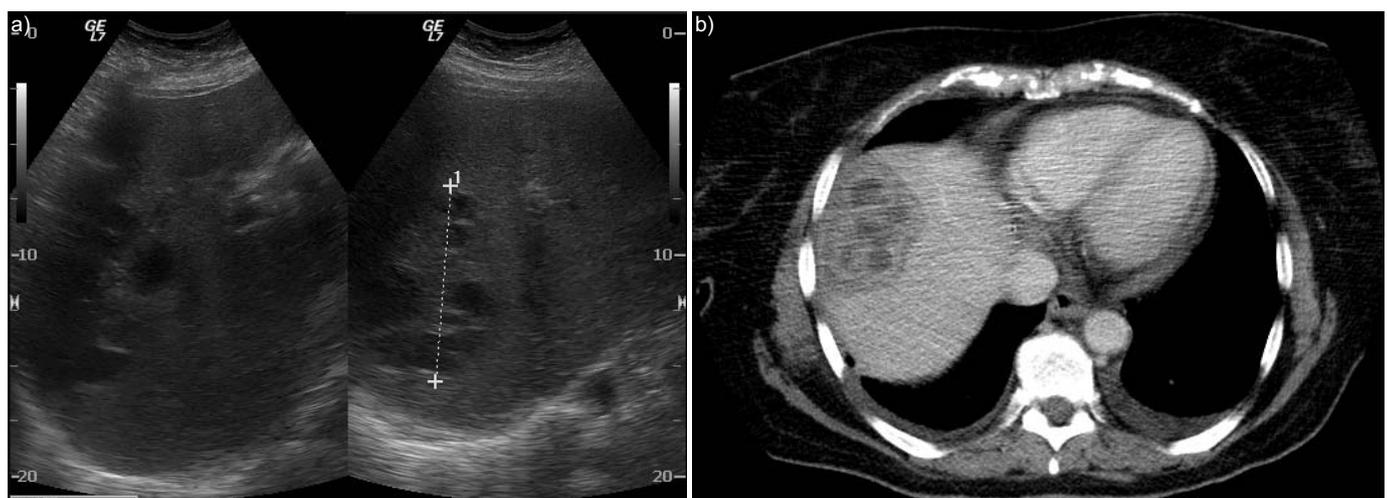

FIGURE 2. Amoebic hepatic abscess. a) Echographic image showing multiple cavities. b) Computed tomography scan shows that the lesion is located at the top of the right hepatic lobule, very near to the diaphragmatic cupola.

FIGURE 3. Thoracic amoebiasis. Thorax radiograph showing elevation of the right diaphragmatic cupola, and extensive infiltrates.

of the presence of mobile forms of the micro-organism in secretions of the respiratory tract, and their subsequent cultivation in an appropriate medium, are procedures used in the diagnosis.

With regard to *T. tenax*, present in the oral cavity and frequently found in dental plaque, this is the species most frequently described in the literature reviewed. The appearance of this micro-organism is the most frequent cause of the development of a pulmonary pathology [57]; factors that predispose towards its development are poor dental hygiene, malnutrition, alcoholism and prior debilitating or pulmonary diseases (carcinoma, abscess, bronchiectasis, *etc*.) [58, 59]. In these cases, pleural discharge is the most common complication [60–63], and the presence of mobile forms is observed in fresh preparations of the fluids obtained. Giemsa staining is also quite useful. In other kinds of samples, such as bronchoalveolar lavage, its presence has been described, together with numerous eosinophils, in a patient with a history of asthma [64]. Identification of the species is possible using molecular biology techniques [65, 66].

T. hominis, also known as *Pentatrichomonas hominis*, is the species that appears least often in the literature [67, 68]. As it is a micro-organism located in the intestine, it is supposed that it may reach the respiratory apparatus by aspiration or through a bronchoenteral fistula. A necrotising pulmonary abscess and pleural effusions are the pathological situations described.

In the genera Tritrichomonas and Tetratrichomonas there are many species that may be pathogenic for various animals (sheep, pigs, cats, birds, *etc*.). Of these, *Tritrichomonas foetus* and *Tetratrichomonas gallinarum* are two of the most representative. It has recently been suggested that some of these species originating in animals may have become adapted to humans. Proof of this are two studies that, by means of biological tests, have demonstrated genetic sequences of these protozoa in samples from the human respiratory apparatus [69, 70]. These findings have been recognised as opening up an important new field of research [3].

FIGURE 4. *Trichomonas vaginalis*: two Giemsa-stained trophozoites. Scale bar=15 μm.

Genus Lophomonas

Protozoa belonging to this genus are found as symbionts in the intestine of certain arthropods, such as termites and cockroaches, contributing to the process of digestion of some materials, such as cellulose.

In the published literature, two studies from China are mentioned in which protozoa of the Hypermastigida order are related to pulmonary pathology, responding to metronidazole therapy [71, 72]. Furthermore, the species *Lophomonas blattarum* is implicated in four cases of patients undergoing renal transplant who developed pulmonary pathology; in all these cases, therapeutic response with metronidazole was also demonstrated [73].

Protozoa forms not so far catalogued but which, from their morphological and staining characteristics, are presumed to be flagellated protozoa (fig. 5a and b), have been described in the sputum of asthmatic patients [74], nasal secretions of patients with allergic rhinitis [75] and sputa of immunodepressed patients, especially those with AIDS [76, 77].

A striking fact is the finding of similar flagellated protozoa in intestinal extracts of dust mites and cockroaches, establishing a possible nexus between these micro-organisms and respiratory allergy [78–80].

Genus Leishmania

This genus includes a great number of species, noteworthy among which are *L. donovani*, *L. tropica*, *L. major* and *L. infantum* (present in the Mediterranean basin), with a biological cycle integrated by an extracellular form or promastigote in the intestinal tract of phlebotomes (small insects similar to mosquitoes), and an intracellular form or amastigote that mainly attacks the cells of the reticuloendothelial system. Leishmaniasis is endemic in regions of Asia, Africa, Central and South America and the Mediterranean area of Europe [81].

There are three forms of leishmaniasis: cutaneous, mucocutaneous and visceral (kala-azar). Many of the forms that affect the viscera (lungs, larynx, gastrointestinal tract, *etc*.) are considered opportunistic infections in cases of AIDS [82, 83].

In animal experimentation models, the pulmonary lesion caused by Leishmania spp. is characterised by chronic diffuse interstitial pneumonitis with thickening of the alveolar septa due to the depositing of collagen and a cellular exudates
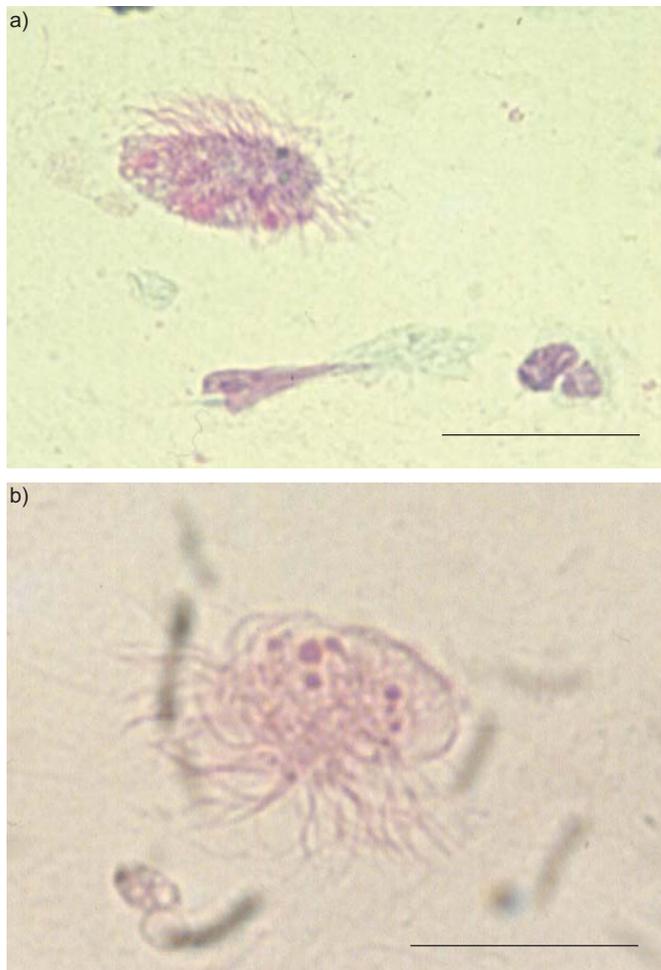
a)

b)

FIGURE 5. Multiflagellated protozoa on sputum smears from AIDS patients (Papanicolaou stain). Scale bars=20 μm.

formed principally by macrophages, lymphocytes and plasmatic cells [84]. A similar pulmonary pathology involving foci of septal fibrosis and interstitial pneumonitis with a predominance of mononuclear cells has also been described in humans [85]. Other forms in which the disease presents itself are granulomatous inflammation of the bronchial mucosa and mediastinal lymph nodes [86], and the development of pleural effusions with the presence of the intracellular protozoon in macrophages [87, 88]. In other types of samples of the respiratory apparatus, such as transbronchial biopsy [89] and bronchoalveolar lavage [90, 91], the presence of Leishmania amastigotes has also been detected.

Infection of the mucosa of the larynx is another complication of leishmaniasis. This infection is frequently encountered in AIDS patients [92, 93], although cases have also been described in immunocompetent patients [94]. In these latter cases, differential diagnosis with respect to neoplasic processes has been proposed [95]; apart from the histological findings, the demonstration of specific DNA sequences in the tissues affected by means of molecular biology techniques is also very useful [96].

Direct observation of the parasite (fig. 6) is one of the best methods of diagnosis and, in the case of visceral leishmaniasis,

is usually carried out in bone marrow aspirates by means of haematological stains. Nowadays, molecular biology techniques are an alternative that should also be considered.

The use of medicines such as pentavalent antimonial derivatives is one of the treatments of choice in leishmaniasis, although other substances such as paromomycine and liposomal amphoterycine B are effective alternatives [97].

Genus Trypanosoma

The Trypanosoma genus comprises various species of haemoflagellated protozoa transmitted to man by the bite of flies and bedbugs. Their names reflect the region of the planet they inhabit: *T. brucei rhodesiense* and *T. brucei gambiense* (Africa), responsible for sleeping sickness or African trypanosomiasis; and *T. (Schizotrypanum) cruzi* (South America), responsible for Chagas' disease or trypanosomiasis Americana. It is the latter that may affect the respiratory apparatus, either in organs such as the heart and oesophagus [98], or during pregnancy through the placenta (the congenital form of the disease).

In a wide study of *post mortem* cases both myocarditis and megaoesophagus were the cause of a series of pulmonary complications, such as pleural effusion, thromboembolism, aspiration pneumonia, pulmonary abscess, bronchiectasis and tuberculosis [99].

In experimental animals it has been demonstrated that parasitaemia due to *T. cruzi* is the cause of pneumonitis [100], with changes in the pulmonary parenchyma, such as thickening of the alveolar walls due to proliferation of type II pneumocytes, macrophages and mononuclear inflammatory infiltration with oedema. The alveolar spaces contain liquid, fibrin, hyaline membranes and erythrocytes. The presence of micro-organisms was observed in the walls of the bronchia accompanied by an inflammatory reaction. Micro-organisms also appeared in the walls of the large vessels.

In cases of the congenital form of the disease, pneumonitis is the most frequent lesion, with the presence of amastigotes in lungs, placenta membranes and the umbilical cord [101].
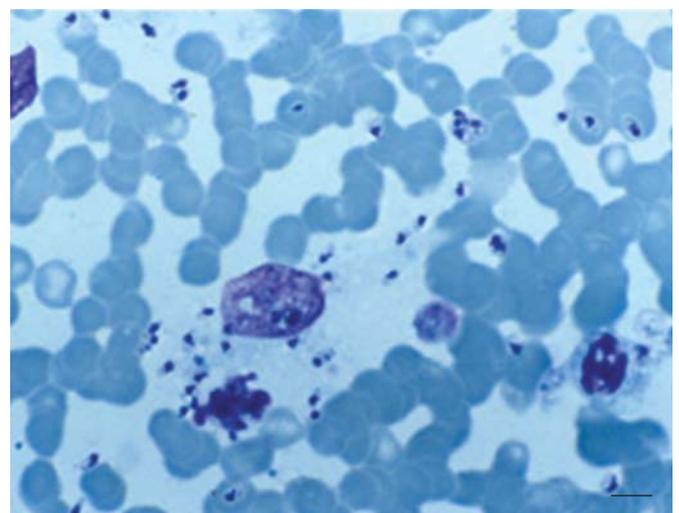

FIGURE 6. *Leishmania infantum*: Giemsa-stained amastigotes in a smear from the bone marrow. Scale bar=7 μm.

During the acute phase of Chagas' disease, numerous parasites that can be detected by direct parasitological tests appear in peripheral blood. Microscopic observation of fresh blood can easily reveal the presence of the parasite, thanks to its motility.

Blood smears in fine thin film and thick drop, appropriately stained, allow the typical morphological characteristics of the parasite to be observed (fig. 7). If the degree of parasitaemia is low, it is vital to use concentration methods such as the microhaematocritic. Xenodiagnosis (visualisation of the parasite in the faeces of the vector) and haemocultivation are the established indirect methods, the sensitivity of which depends on the degree of patient parasitaemia. Moreover, there is widespread use of serological and molecular methods.

Nifurtimox, a derived from nitrofurane, and benznidazole (a nitroimidazole) are practically the only medicines used to treat Chagas' disease.

Phylum Apicomplexa (Sporozoa)
These protozoa are intracellular parasites with one exclusive characteristic, the presence of what is known as the ''apical complex'', an intracytoplasmic structure related with the adhesion to and invasion of the host cells. Various genera of this phylum may act on the respiratory apparatus.

Genus Cyclospora
Of the various species belonging to this genus, only *Cyclospora cayetanensis* is pathogenic for man; the micro-organism is acquired following the ingestion of sporulated oocysts through contaminated food and water [102]. In immunocompetent individuals, depending on their immune state, infection may be asymptomatic or provoke a self-limited diarrhoea episode (cases frequently occur following visits to tropical countries). In immunodepressed patients, especially in AIDS cases, the intestinal episode is more serious and tends to become chronic.

Although effects of Cyclospora on the respiratory apparatus have not so far been identified, two cases have been documented of the presence in sputum of oocysts, corresponding to the species *C. cayetanensis*, with concomitant infection by tuberculosis in both cases [103, 104].

Microscopic identification of Cyclospora oocysts (round structures 7–10 μm in diameter and with a visible wall) can be carried out either in fresh samples or using special staining techniques (trichromic, modified Ziehl–Neelsen, safranine, calcofluor white, *etc.*). Detection methods based on the PCR technique also exist. The treatment of choice is the combination of trimethoprim plus sulfamethoxazole.

Genus Cryptosporidium
The genus Cryptosporidium comprises 13 species of intracellular enteric protozoa widely distributed in animals, especially birds, cattle and sheep. In humans, the species most often detected are *C. parvum*, *C. hominis* and *C. meleagridis* [105]. The infection is acquired by the ingestion of oocysts (fig. 8) through water and food contaminated with faecal matter, contact with animals, or from person to person.

The principal clinical expression of cryptosporidiosis is watery diarrhoea of variable duration, the process being self-limiting in immunocompetent persons, and tending to become chronic in immunodepressed patients, among whom it may prove fatal. It is among this latter group that clinical forms of extraintestinal repercussions may be observed [106].

All the documented cases of invasion of the respiratory apparatus by Cryptosporidium correspond to immunodepressed patients, the principal cause being the appearance of AIDS [107–116]. Other processes also exist, such as those corresponding to malign haematological pathologies [117] and bone marrow transplant recipients [118, 119]. Although the mechanism by which Cryptosporidium colonises the respiratory apparatus is not clear, aspiration of gastric content and haematogenous dissemination facilitated by macrophages originating in an intestinal focal point have been proposed as possible routes [120].

Both clinical observation and radiography tend to be imprecise, the symptoms most often referred being persistent
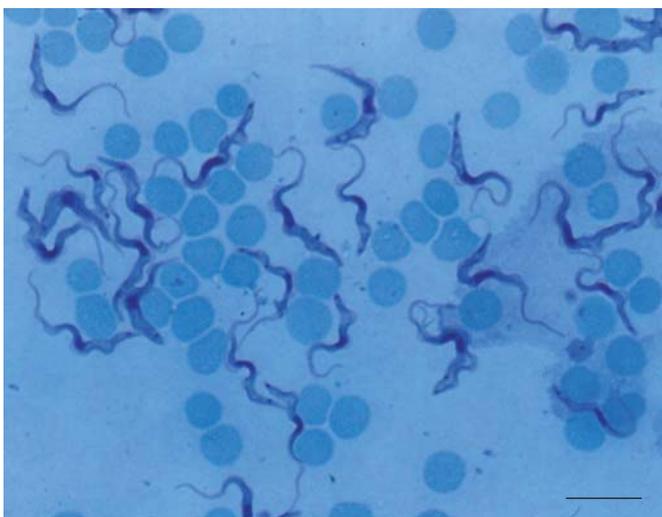
FIGURE 7. *Trypanosoma brucei gambiense*: Giemsa-stained trypomatigotes in a thin blood film. Scale bar=14 μm.
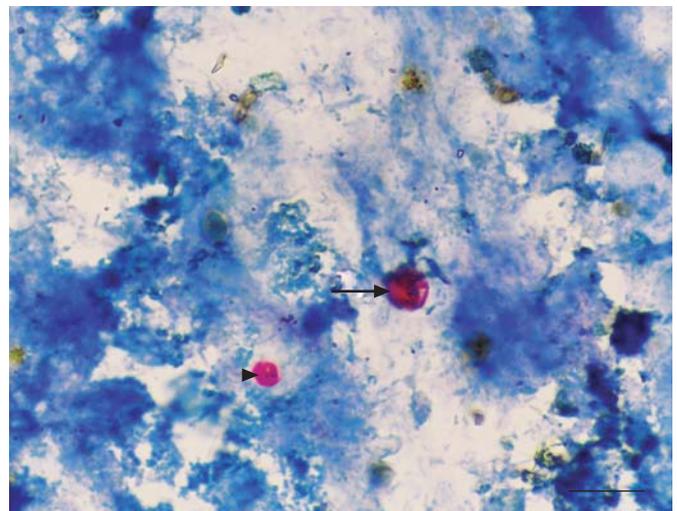
FIGURE 8. *Cryptosporidium* sp. oocysts (arrowhead) and *Cyclospora cayetanensis* oocysts (arrow) in red in a modified acid-fast stained faecal smear. Scale bar=10 μm.

productive cough, fever, dyspnoea and tachypnoea, in some cases requiring mechanical ventilation due to respiratory failure and hypoxaemia. On chest radiographs, the most commonly observed pattern is that of interstitial pneumonia.

Studies in experimental animals subjected to immunosuppression have demonstrated that Cryptosporidium may directly damage the respiratory epithelium [121–123], especially noteworthy being the presence of tracheitis with scaly metaplasia and submucous lymphocyte infiltration, peribronchial glandular hyperplasia, loss of cilia and both nuclear and cytoplasmatic changes. The occupation by a purulent exudate in the bronchial cavities, with numerous micro-organisms adhering to the epithelial surface, is also described. Thickening of the intra-alveolar walls with an inflammatory infiltrate based on macrophages and lymphocytes was observed and also, in the alveolar spaces and even in macrophages, free micro-organisms.

These same alterations have been described in *post mortem* findings from patients who died of respiratory failure; data also exist regarding diffuse alveolar damage, especially hyperplasia of type-II pneumocytes and interstitial fibrosis.

Despite this, even when the autopsy confirms the presence of Cryptosporidium in lung tissue, it is sometimes difficult to confirm its implication in death owing to the fact that other pathogens are also present in the bronchial tree [124].

The presence both of noncystic forms of the protozoon (sporozoites and merozoites), free or in macrophages, and of oocysts (with a wall, spherical in shape and having a diameter of 4–6 μm) can be demonstrated in samples such as sputum, tracheal aspirate and bronchoalveolar lavage [125]. Staining methods such as the modifications of Kinyoun and Ziehl–Neelsen and staining with auramine fluorescence are useful here. Molecular biology techniques help in determining the species of the parasite [126].

Paromomycin and azithromycin are the drugs of choice for the treatment of this protozoosis [127], with inhalation a route to be borne in mind [128].

Genus Toxoplasma

Toxoplasma gondii, an obligate intracellular protozoon, is the agent causing toxoplasmosis, an infectious disease found worldwide that affects humans and many animal species alike, cats being the definitive host of the parasite. In its life cycle, *T. gondii* adopts three forms: oocyst, found in the intestine of the cat and excreted to the exterior in its faeces; tachyzoite, the mature form responsible for parasitaemia; and bradyzoite, which forms dormant intracellular cystic aggregates and is responsible for the immune state of the individual.

In healthy persons, toxoplasmosis causes asymptomatic infection or subclinical forms (fever and lymphadenopathies). The most serious form is congenital toxoplasmosis (contracted by the mother during pregnancy), which affects immunodepressed women and is responsible for systemic infections.

Although pulmonary toxoplasmosis may develop in immunocompetent humans [129], there is in most cases a severe deficiency in the immune system. It is the second or third most frequent systemic infection among immunodepressed persons

after brain and cardiac locations [130, 131]. Apart from AIDS [132–136], other processes, such as malign haematological pathologies and organ transplants, are also important causes of pulmonary toxoplasmosis [137–143]. In the majority of these cases, the most widely accepted hypothesis regarding the development of pulmonary toxoplasmosis is the reactivation of a previously latent infection [144, 145], the levels of interferon-γ and the activity of alveolar macrophages being important causative factors. Furthermore, experimental studies on animals have revealed changes in the levels of interleukins segregated by T-helper type-1 lymphocytes in the lung in cases of reactivation of the infection by *T. gondii* [146].

The most frequent clinical symptoms are fever, cough, laboured breathing, tachypnoea and dyspnoea, with this latter, in some cases, leading to hypoxaemia with acute respiratory distress syndrome, which is one of the main causes of death among the patients [147–149].

Radiological examination usually reveals two patterns: the typical bilateral diffuse infiltrate (fig. 9), indistinguishable from other processes such as pneumonia caused by *Pneumocystisis jiroveci*; and a thick nodular bilateral infiltrate, which may be of use in differential diagnosis [150]. In general, these effects reveal interstitial pnemonitis and alveolar condensation. The existence of concomitant pleural effusions is another of the effects described. The anatomopathological examinations carried out, either in pulmonary biopsies or during necropsies, show lesions relating to the state and intensity of the infection [135]. Macroscopically, the lungs appear congested, with petechial haemorrhages and areas of consolidation. Histopathology shows interstitial pneumonitis with inflammatory lymphocyte infiltrate, diffuse alveolar
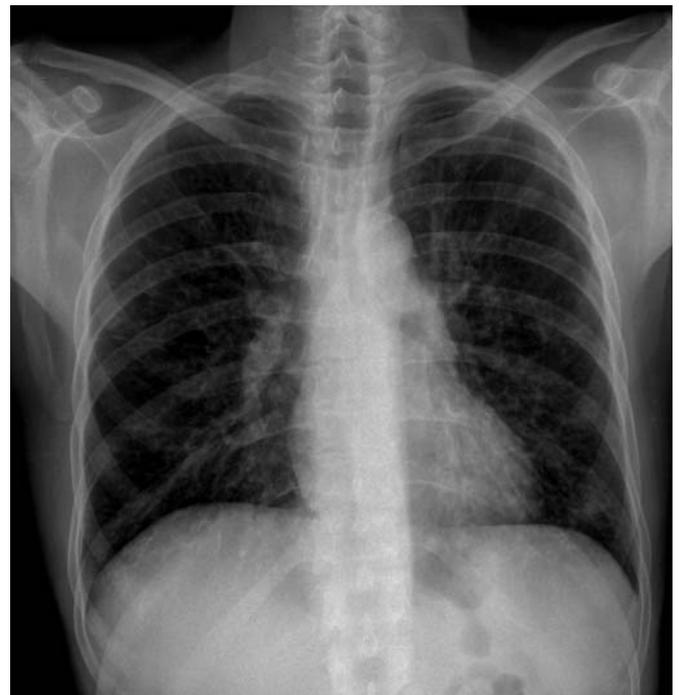

FIGURE 9. Pulmonary toxoplasmosis in a bone marrow transplanted patient, showing bilateral diffuse infiltrates.

damage with fibrinous alveolar exudates and the formation of alveolar hyaline membranes. There are usually numerous alveolar macrophages, which contain cysts of the parasite. Necrotising pneumonia appears as a more advanced lesion, characterised by extensive areas of parenchymatose necrosis and the presence of numerous tachyzoites, both extracellular and intracellular. In the cytological smears prepared from pleural effusions and stained with the May–Grünwald–Giemsa technique, the visualisation of many tachyzoites located extracellularly and intracellularly of polymorphonuclear leukocytes and macrophages is described [151, 152].

Various diagnostic procedures permit visualisation of the parasite in samples from the respiratory apparatus, one of the most significant being bronchoalveolar lavage [153–156] with Giemsa staining of the samples. In the sputum *T. gondii* also appears, this being an alternative diagnostic route if invasive techniques cannot be carried out [157]. In cases in which infection is suspected, or in which the micro-organisms are not observed, techniques such as PCR may be of great use [158, 159].

The most appropriate therapeutic measures are the combination of pyrimethamine and sulfadiazine administered to prevent haematological complications, or the substitution of sulfadiazine by clindamycin. Alternatives to these combinations are azithromycin and doxycycline. Apart from therapy, in immunodepressed patients primary prevention and appropriate prophylaxis against *T. gondii* may significantly mitigate pulmonary repercussions [160].

Genus Plasmodium

This genus comprises the intracellular protozoa that are responsible for malaria. The disease is endemic in >90 countries, affects 200–300 million people each year and, each year, is responsible for the death of ~3 million. It is, therefore, one of the diseases with highest morbidity and mortality. Four of the species belonging to this genus affect humans: *P. falciparum*, *P. malariae*, *P. ovale* and *P. vivax*. The disease is transmitted by the bite of the female Anopheles mosquito, and the Plasmodium has a life cycle consisting of an asexual stage in human hosts, with extraerythrocytic and intraerythrocytic phases, and a sexual stage that develops in the mosquito.

In the current authors' environment, malaria is one of the principal imported acute diseases with pulmonary manifestations [161, 162]. *P. falciparum* and *P. vivax* (fig. 10) are the species that most frequently affect the lungs, while *P. ovale* and *P. malariae* are the rarest [163]. The groups principally affected are children, pregnant women and travellers to countries where the disease is endemic [164].

Pulmonary oedema is the principal manifestation of the effects of malaria on the lung [165], especially in cases involving *P. falciparum* and, to a lesser extent, *P. vivax* [166]. The increased permeability of the alveolar capillaries appears to be the principal mechanism by which the plasmatic liquid fills the alveolar spaces [167]. *In vitro* experimental models have demonstrated that the adherence of the infected erythrocytes to the vascular epithelium, through various molecules (intercellular adhesion molecule-1, E-selectin, vascular cell adhesion molecule-1, *etc.*), is one of the main factors provoking capillary damage with subsequent increase in permeability [168, 169]. Another frequent pulmonary complication of malaria is acute
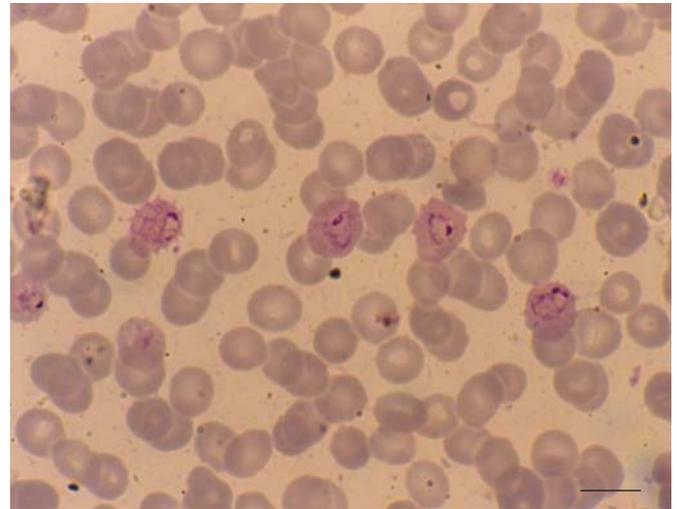

FIGURE 10. *Plasmodium vivax*: infected red blood cell containing a stained trophozoite showing the typically amoeboid appearance in a Giemsa thin blood film. Scale bar=7 µm.

respiratory distress syndrome, in which both *P. falciparum* [170–172] and *P. vivax* [173–179] are implicated.

The clinical impact of malaria on the lung may range from mild, with fever, cough, dyspnoea, expectoration and thoracic pain [180, 181], to serious respiratory insufficiency requiring admittance to an intensive care unit [182].

Radiological examination reveals the presence of small diffuse and bilateral infiltrates, with a marked increase of the vascular net and areas of lobar consolidation [183]. Associated pleural discharge may also be observed.

In the anatomopathological examination, macroscopically, the lungs appear congested and oedematose, with numerous haemorrhagic focal areas. The coexistence of pleural and/or pericardial discharge may be observed. In histological sections, pleural oedema, capillary congestion, hyaline membranes and thickening of the alveolar septa are evident. The presence of a brown pigment (haemozoin, or malarial pigment) may be observed in the interior of the alveolar macrophages. A case of obliterating bronchiolitis with organisational pneumonia associated to *P. vivax* is described in the literature, with therapeutic response to corticoids [184].

Visualisation of the protozoon through microscopic examination of the blood is the diagnostic method of choice, although there may be recourse to other diagnostic techniques, including PCR, for a correct identification of the species [185].

As well as depending on the appropriate support treatment to control haemodynamic and respiratory function, antimalarial drug therapy depends on a series of factors, such as the type of species, clinical state of the patient and the susceptibility of the parasite to the drugs, especially in relation to the area where the infection was acquired. One noteworthy aspect is the pulmonary toxicity that may be produced by the use of mefloquine, with the development of diffuse alveolar damage [186–188].
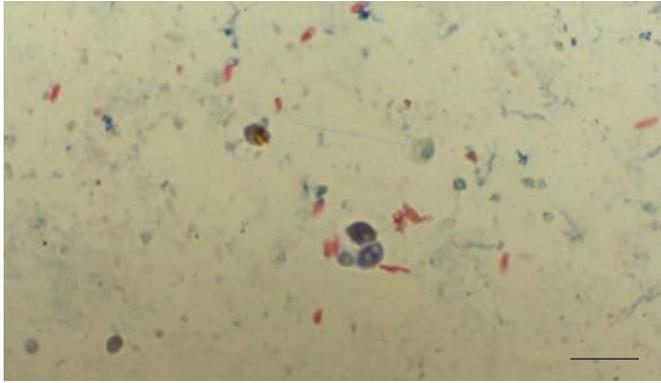
FIGURE 11. *Encephalitozoon hellem*: spores stained with a modified trichrome stain in bronchoalveolar lavage. Scale bar=6 μm.

Genus Babesia

The Babesias are intraerythrocytic protozoa of which there exist various species, fundamentally *B. divergens* and *B. microti*. Their principal reservoirs are cattle and wild rodents. Humans acquire the infection (babesiosis), characterised fundamentally by the presence of fever and haemolysis, through tick bites. Among the factors of risk for systemic infection are immuno-suppression, advanced age and antecedents of splenectomy. Babesiosis is quite a rare disease, in which the effect on the lung is, as is the case with malaria, a consequence of a systemic inflammatory response [189], which, in some cases, may be secondary to the antiprotozoa therapy employed [190, 191]. Among the clinical manifestations are fever, cough and laboured breathing, with noncardiogenic pulmonary oedema being the most frequent development [192]. Among the possible causes of this pulmonary complication are the lack of deformability of the infected red blood cells as they pass through the pulmonary capillaries and an increase in their adhesiveness to the vessel walls.

In the literature there are three cases in which acute respiratory distress syndrome developed as a complication of the disease [193, 194], with mechanical ventilation being required due to respiratory failure and hypoxaemia. In the radiological examinations, bilateral infiltrates with an alveolar pattern and thickening of the septa are described [195]. In one of the cases, the *post mortem* findings showed marked congestion and pulmonary consolidation; in the histological sections, hyper-plasia of type II-pneumocytes, interstitial oedema and foci of formation of hyaline membranes are observed.

The diagnosis of babesiosis is established with the observation of intraerythrocytary parasites with characteristic morphology (gemmated or ''Maltese cross'' shapes) in peripheral blood. Techniques such as PCR allow a more specific diagnosis. The treatment of choice is the joint administration of clindamycin and quinine sulphate.

Phylum Microspora

The protozoa belonging to this phylum are intracellular obligate parasites (they lack organs such as mitochondria and Golgi apparatus). Their life cycle consists of an infective extracellular phase and a multiplication phase in the host cell, with spores, made up of small encapsulated elements (1–4 μm
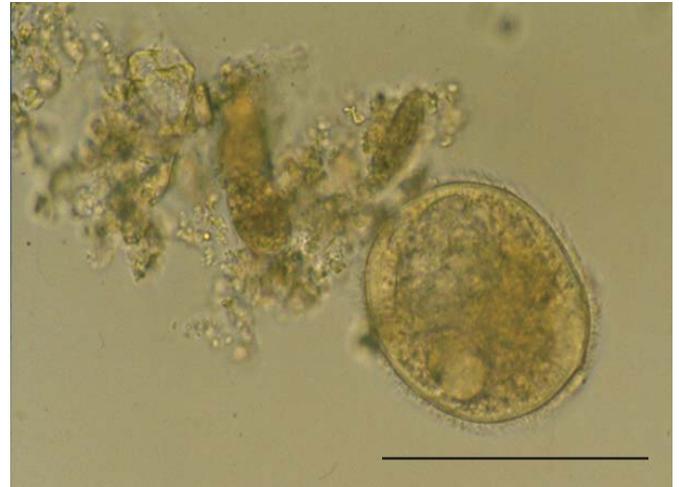

FIGURE 12. *Balantidium coli*: trophozoite in wet mount stained with iodine. Scale bar=200 μm.

in diameter, oval morphology), as the infecting forms [196]. Of the eight kinds implicated in human pathology, the most frequent species are *Enterocytozoon bieneusi*, *Encephalitozoon cuniculi*, *Encephalitozoon hellem* and *Encephalitozoon intestinalis*.

In an immunocompetent person, microsporidiosis may be an asymptomatic infection or cause mild diarrhoeic episodes, while in immunodepressed patients the infection acquires particular importance, the most frequent form being gastro-intestinal, although multiorganic repercussions also exist [197, 198]. Oral, ocular, sexual and respiratory transmission routes are described, the latter being confirmed by the observation of micro-organisms in the sputum and in the tracheobronchial tree [199, 200].

All the documented cases of pulmonary microsporidiosis correspond to immunodepressed patients, the predisposing factors being AIDS [201–204] and bone marrow transplant recipients [205–207]. Prominent among the possible mechanisms by which the micro-organisms colonise the respiratory appara-tus are inhalation, regurgitation, orofaecal contamination and haematogenous dissemination from an intestinal focus [208].

Among the clinical manifestations of infection of the respira-tory apparatus by microsporidia are rhinosinusitis [209], fever, persistent cough, dyspnoea and acute respiratory distress that, in some cases, developed before the patients' death due to cardiorespiratory failure.

Noteworthy in radiographic examinations is the presence of infiltrates and bilateral pulmonary opacities, along with the existence of areas of extensive consolidation.

The samples in which the micro-organism have been identified are nasal secretions, sputa, tracheobronchial aspirate and bronchoalveolar lavage (fig. 11), in which it was possible to carry out staining (Weber's modified trichromic and calco-fluor), immunofluorescence, electron microscopy and specific cell cultures [210, 211].

In those cases in which an anatomopathological examination was performed, the biopsies show bronchiolitis with epithelial

inflammatory infiltrate due to lymphocytes. In the necropsies, the lungs presented multiple areas of abscess, especially in the subpleural area.

Although therapy depends on the species involved and the immunological state of the patient, albendazole and fumagaline are two of the compounds habitually used.

Phylum Ciliophora (ciliates)

The fundamental feature of these protozoa is the presence of cilia, along the whole of the cellular membrane or at specific locations, which are used both for movement and for food capture. Until now, the only species known to produce pathology in humans is *Balantidium coli*; the only species in this genus. This is one of the largest protozoa (50–300 μm in length), with an ovoid body covered with cilia (fig. 12). Its principal natural habitat and reservoir is the large intestine of the pig, although it also inhabits that of humans. Although there is controversy as to the way this protozoon is acquired by humans, an important factor would seem to be some relation with pigs. Even so, person-to-person transmission should also be considered. Infection in humans fundamentally affects the colon, provoking clinical situations of variable intensity, ranging from asymptomatic forms to severe diarrhoea with ulceration of the mucosa.

Pulmonary infection by *B. coli* is quite rare, with few cases described in the literature. Among the predisposing factors are, fundamentally, immunosuppression [212, 213] and contact with pigs [214, 215], although a case has also been described of a patient with antecedents of asthma who was not immunodepressed [216]. Although the mechanism by which the lungs are affected is not completely clear, haematogenous and lymphatic dissemination by ulceration of the intestine and also, in the case of perforation, through the diaphragm, seems probable. In the majority of cases, the following are among the clinical manifestations: fever, nonproductive and persistent cough, dyspnoea of variable intensity (one of the patients required assisted respiration) and thoracic pain. Noteworthy in the radiological examinations was the presence of diffuse and bilateral interstitial infiltrates. In the examination of fresh samples, whether obtained by aspiration or by bronchoalveolar lavage, the presence of numerous trophozoites is described.

Metronidazole or tetracycline therapy seems to be effective.

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