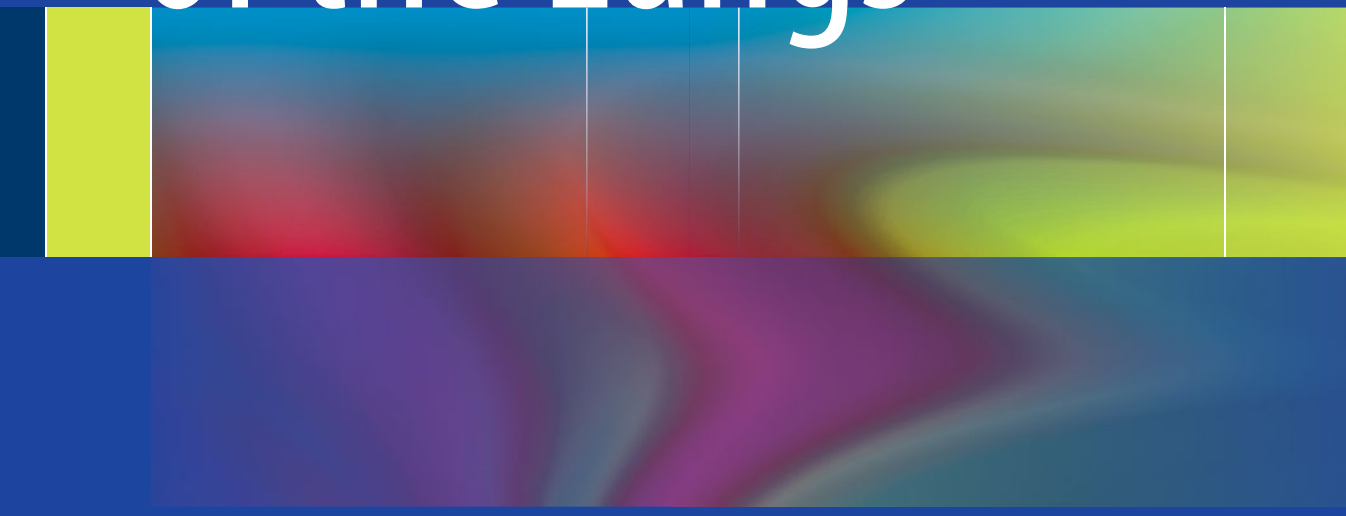
Roberto Barrios
Abida K. Haque
Editors

Parasitic Diseases of the Lungs



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Preface

It has become widely recognized over the past two decades that many patients with parasitic infections of the lung do not receive an accurate diagnosis in a timely fashion; this applies especially to immunosuppressed patients. Although some parasitic diseases of the lung are uncommon and seen in endemic areas, the clinician should keep in mind that with immigration and widespread travel, even uncommon parasitic diseases can be seen in any latitude. There are few books dealing directly with parasitic diseases of the respiratory system. This book represents an attempt to summarize the natural history, pathogenesis, molecular mechanisms, and diagnostic features of parasitic diseases involving the lungs. It is quite evident that the present accretion of knowledge makes it impossible to compress all available information in a small volume. The question is what to include and what to delete. We tried to offer our readers a practical approach to the diagnosis of parasitic diseases involving the lungs and at the same time to provide a general background of the current molecular biology and immunological mechanisms that cause tissue injury. We hope that the present volume can serve as a reference tool to the clinician, radiologist, and the practicing pathologist. The first chapters will help the clinician to review general concepts of pulmonary parasitic diseases. Chapters on molecular biology and immunology will provide a good review of the current literature on these topics. Finally, the practicing pathologist will find concise information on the morphology of the most common pathogenic organisms and of structures that resemble organisms but are in fact artifacts during tissue processing and sectioning or the result of endogenous or exogenous particles. This is the purpose of the chapter entitled "Pseudoparasitic Structures" that we hope will be of help to the pathologist to avoid common diagnostic errors.

Last but not least, the authors would like to express their gratitude to Ms. Connie Walsh and Ms. Sandra Lesny of Springer for their great help and patience during the preparation of this book.

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Vannan K. Vijayan

1.1 Introduction

Pulmonary parasitic lung diseases are commonly diagnosed in countries where the prevalence of parasitic infection is high. However, there is an increase in the number of patients diagnosed as parasitic lung diseases recently, even in countries of low prevalence of parasitic infection which demands an awareness of such diseases in these countries. This increase in diagnosis in countries with low prevalence with parasitic infections has been attributed to an increase in the numbers of immunosuppressed individuals due to various reasons, organ transplantations and global travel [1]. The parasites can cause a wide spectrum of lung diseases varying from mild self-limiting bronchitis to life-threatening acute respiratory distress syndrome [2]. In addition, parasitic lung diseases may mimic diseases such as bacterial pneumonias, pulmonary tuberculosis, bronchial asthma, lung cancer, interstitial lung disease, and pulmonary hypertension. Both protozoal and helminthic parasites can cause lung diseases and helminthic lung infections are important causes of eosinophilic lung diseases [3]. Though the treatment of parasitic lung diseases is with specific antiparasitic drugs, the physicians treating such diseases should be competent in tackling the specific issues related to lung injury and sequel that may follow such infections. The lung diseases that may result from infections with parasites are listed in Table 1.1 [4].

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1.2 Protozoal Parasites

The important protozoal parasites that cause pulmonary diseases are *Entamoeba histolytica*, *Leishmania donovani*, malarial parasites (*Plasmodium vivax*, *P. falciparum*, *P. malaria*, *P. ovale*, *and P. knowlesi*), *Babesia* spp. (*Babesia microti* and *Babesia divergens*), and *Toxoplasma gondii*.

1.2.1 Pulmonary Amebiasis

1.2.1.1 Clinical Diagnosis

Amebiasis results from ingestion of mature *Entamoeba histolytica* cysts in fecally contaminated food, water or from hands. Infection with *Entamoeba histolytica* can lead to intestinal colonization, colitis or extraintestinal manifestations resulting from the hematogenous spread of infection from the intestine. About 90 % of intestinal colonization is with nonpathogenic species, *Entamoeba dispar* and *Entamoeba moshkovskii*. Invasive amebiasis occurs in 10 % of persons colonized with *E. histolytica*. Patients with amebic colitis present with several-week history of cramping abdominal pain, weight loss, and watery or bloody diarrhea. Extraintestinal amebic infection can manifest as amebic liver abscess,

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Table 1.1 Pulmonary diseases caused by parasitic infections

Diseases	Parasites
I. Protozoa	
1. Pulmonary amebiasis	*Entamoeba histolytica*
2. Pulmonary leishmaniasis	*Leishmania donovani*
3. Pulmonary malaria	*Plasmodium vivax*
	Plasmodium falciparum
	Plasmodium malariae
	Plasmodium ovale
	Plasmodium knowlesi
4. Pulmonary babesiosis	*Babesia microti*
	Babesia divergens
5. Pulmonary toxoplasmosis	*Toxoplasma gondii*
II. Helminths	
(a) Cestodes	
1. Pulmonary hydatid disease	*Echinococcus granulosus*
	Echinococcus multilocularis
(b) Trematodes	
1. Pulmonary schistosomiasis	*Schistosoma haematobium*
	Schistosoma mansoni
	Schistosoma japonicum
2. Pulmonary paragonimiasis	*Paragonimus westermani*
(c) Nematodes	
1. Pulmonary ascariasis	*Ascaris lumbricoides*
2. Pulmonary ancylostomiasis	*Ancylostoma duodenale*
	Necator americanus
3. Pulmonary strongyloidiasis	*Strongyloides stercoralis*
4. Tropical pulmonary eosinophilia (pulmonary filariasis)	*Wuchereria bancrofti*
	Brugia malayi
	Brugia timori
5. Pulmonary dirofilariasis	*Dirofilaria immitis*
	Dirofilaria repens
6. Visceral larva migrans	*Toxocara canis*
	Toxocara cati
7. Pulmonary trichinellosis	*Trichinella spiralis*

Source: Reprinted from Vijayan [4], with permission

splenic abscess, brain abscess, empyema, and pericarditis. Nearly 80 % of cases of amebic liver abscesses occur in the right lobe. The most common complication of amebic liver abscess is rupture into the pleural space resulting in pleuropulmonary amebiasis [5]. The main symptoms in pleuropulmonary amebiasis are fever, cough, hemoptysis, right upper quadrant abdominal pain, and chest pain. Some patients may present with respiratory distress and shock. Lung abscess, hepatobronchial fistula, and bronchopleural fistula with pyopneumothorax have also been reported. Expectoration of anchovy sauce-like pus indicates amebiasis [6]. The findings of elevated hemidiaphragm, tender hepatomegaly, pleural effusion, and basal pulmonary involvement are suggestive of pleuropulmonary amebiasis. Amebiasis can be suspected in patients with a history of immigration from or travel to developing countries and many patients give history of dysentery and alcoholism.

1.2.1.2 Laboratory Diagnosis

Amebiasis is commonly diagnosed by microscopy and cysts or motile trophozoites can be identified on a saline wet mount of a stool specimen [7]. Microscopic examination of fresh stools, sputum or pleural pus, rectal smears or rectosigmoidoscopy materials, pus from liver abscesses, and colonic biopsy samples may reveal motile trophozoites, even though it is a relatively specific test but is not sensitive for the identification of *E. histolytica*. The presence of ameba in the stool does not indicate that the disease is due to pathogenic *E. histolytica* as two other nonpathogenic species found in humans (*E. dispar* and *E. moshkovskii*) are indistinguishable morphologically [8, 9]. This method can, therefore, give false-positive results if *Entamoeba dispar* or *Entamoeba moshkovskii* infection is present. However, it has been reported recently that several different genotypes of *E. dispar* can be potentially responsible for tissue damage similar to that observed with *E. histolytica* [10]. A nonpathogenic *Entamoeba gingivalis* which is present in the oral cavity has to be differentiated from *Entamoeba histolytica* in sputum samples. A combination of serological tests with identification of the parasite by antigen detection by PCR is the best approach to diagnosis.

In vitro culture by inoculation of portions of stool, liver abscess, or empyema fluid into sterile culture media and incubating it at 37 °C is also useful in the diagnosis of amebiasis. The culture media is examined for the growth of amebic trophozoites, which, if present, can be seen on the walls of test tubes or in debris [11]. Antibody detection and antigen detection are other important immunodiagnostic methods. Indirect hemagglutination (IHA) test is used for routine serodiagnosis of amebiasis. Antigen consists of a crude soluble extract of axenically cultured organisms. The enzyme immunoassay (EIA) test detects antibody specific for *E. histolytica* in approximately 95 % of patients with extraintestinal amebiasis, 70 % of patients with active intestinal infection, and 10 % of asymptomatic persons who are passing cysts of *E. histolytica*. Detectable *E. histolytica*-specific antibodies may persist for years after successful treatment, so the presence of antibodies does not necessarily indicate acute or current infection. Specificity is 95 % or higher and false-positive reactions rarely occur. *E. histolytica*-specific antigen detection may be useful as an adjunct to microscopic diagnosis in detecting parasites and to distinguish between pathogenic and nonpathogenic infections. Detection of circulating antigens in the serum has been found to be an important advancement in the diagnosis [12]. Polymerase chain reaction (PCR) assays are useful for the differentiation of *E. histolytica*, *E. dispar*, and *E. moshkovskii* and for genetic typing of isolates [13, 14]. However, these tests are time-consuming and expensive and, hence, are not practical in areas endemic for amebiasis.

1.2.2 Pulmonary Leishmaniasis

1.2.2.1 Clinical Diagnosis

Infection with *Leishmania donovani* causes visceral leishmaniasis and is transmitted by various species of *Phlebotomus*, the sand fly [15]. There are no clinical symptoms and signs that are pathognomonic of visceral leishmaniasis (VL) or kala-azar. The symptoms and signs suggestive of visceral leishmaniasis are irregular fever, weight loss, enlargement of liver and spleen, and anemia. Pneumonitis, septal fibrosis, pleural effusion, and mediastinal adenopathy are reported in patients coinfected with human immunodeficiency virus (HIV) [16]. Leishmaniasis has also been reported in lung transplant patients [17]. *Leishmania* amastigotes can be found in the alveoli, pulmonary septa, and bronchoalveolar lavage (BAL) fluid. Diagnosis of leishmaniasis is by the demonstration of the parasites in bone marrow aspirates and by the identification of specific DNA sequences in tissues by molecular biology techniques [18]. The differential diagnosis of leishmaniasis includes malaria, cirrhosis with portal hypertension, miliary tuberculosis, brucellosis, histoplasmosis, lymphoma, and leukemia [19].

1.2.2.2 Laboratory Diagnosis

The diagnosis of leishmaniasis is based on the microscopical demonstration of *Leishmania* amastigotes in the relevant tissue aspirates or biopsies such as bone marrow, spleen, lymph nodes, or liver, skin slit smears, or in the peripheral blood buffy coat [19]. The smears can be stained with Romnowsky's, hematoxylin-eosin, or immunoperoxidase stains. The amastigote stage seen in clinical samples is known as Leishman-Donovan (LD) bodies. The amastigotes observed in the smears have to be differentiated from "dot"-like structures (e.g., *Histoplasma* spp., platelets) by looking for the characteristic size (2–4 mm in diameter), shape (round to oval), and the internal organelles (the nucleus and kinetoplast). Culture of these specimens on solid NNN medium will demonstrate promastigotes. Immunological methods of diagnosis include indirect fluorescent test (IFA), direct agglutination test (DAT), and enzyme-linked immunosorbent assay (ELISA) to detect antibodies against *Leishmania* [20]. Antigen detection tests are better means of diagnosis of active leishmaniasis [21]. The antigen detection is the ideal test in immunocompromised patients, where antibody response is very poor. PCR is found to be the most sensitive and specific molecular test and is useful in molecular epidemiological studies besides diagnosis [22].

1.2.3 Pulmonary Malaria

1.2.3.1 Clinical Diagnosis

Malaria is caused by the obligate intraerythrocytic protozoa of the genus *Plasmodium* and is primarily transmitted by the bite of an infected female *Anopheles* mosquito. Five species of malarial parasites (*Plasmodium vivax*, *P. falciparum*, *P. malariae*, *P. ovale*, and *P. knowlesi*) infect man [23]. The main symptoms of malaria are periodic fever, chills, malaise, headache, abdominal pain, and vomiting, usually manifesting 10–15 days after mosquito bite. Anemia and splenomegaly are other important findings in malaria. Falciparum malaria is the most deadly type. The pulmonary manifestations range from cough to severe and rapidly fatal non-cardiogenic pulmonary edema and acute respiratory distress syndrome (ARDS) (Fig. 1.1) [24]. Acute lung injury and ARDS have also been reported to occur in infection with *P. vivax* and *P. ovale* [25, 26]. There has been no convincing evidence for the existence of true malarial pneumonitis, and if it occurs, it may be due to viral and secondary bacterial infections. Diffusing capacity was significantly impaired in patients with severe malaria. In addition to ARDS, *falciparum* malaria can cause many other severe complications such as cerebral malaria, acute renal failure, severe anemia, thrombocytopenia, bleeding, and gastrointestinal, hepatic, and metabolic complications [27].

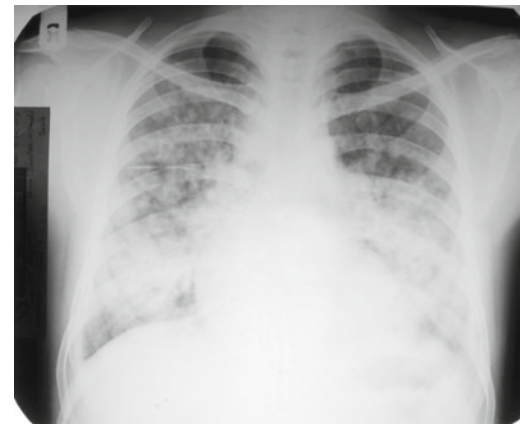

Fig. 1.1 Chest skiagram showing bilateral fluffy shadows in a patient presenting with acute respiratory distress syndrome due to severe falciparum malaria (Adapted from Vijayan and Kilnai [2])

1.2.3.2 Laboratory Diagnosis

Microscopic examination of the Giemsa-stained blood smears is the gold standard for the diagnosis of malaria. Microscopic diagnosis is based on staining thick and thin blood films on a glass slide to visualize the malaria parasite [28]. It is inexpensive, able to differentiate malaria species and quantify parasites. The detection threshold in Giemsa-stained thick blood film has been estimated to be 4–20 parasites/μL. *Plasmodium* species can be correctly recognized in thin blood film. Sometimes malarial parasites cannot be detected in peripheral blood smear, but malaria pigments may be seen in circulating phagocytic leukocytes. This is a pathognomonic sign of recent infection. The parasite count, number of circulating pigment-containing phagocytes, and the presence of late asexual stages of the parasite observed in the blood smear are all positively correlated with a fatal outcome. Bone marrow aspirate can also demonstrate malarial parasites, if thin smears of the peripheral blood do not show the parasites. Quantitative buffy coat (QBC) method involves staining parasite deoxyribonucleic acid (DNA) in micro-hematocrit tubes with fluorescent dyes (e.g., acridine orange) and its subsequent detection by epifluorescent microscopy. The parasite nuclei fluoresces bright green and the cytoplasm appears yellow-orange [29].

Rapid diagnostic test (RDT) is a device that detects malaria antigen in a small amount of blood, usually 5–15 μL, by immunochromatographic assay with monoclonal antibodies directed against the target parasite antigen and impregnated on a test strip [30, 31]. The result, usually a colored test line, is obtained in 5–20 min. Histidine-rich protein 2 (HRP-2) which is specific for *P. falciparum* is the most common malaria antigen targeted [32]. *Plasmodium* lactate dehydrogenase (PLDH) enzyme is the other group of targeted antigens. Monoclonal antibodies against pLDH and aldolase enzymes are available for the detection of *Plasmodium* spp. (pan malaria). HRP-2 often persists in the patient's blood for weeks after successful treatment.

Molecular methods such as PCR allow the specific amplification of a selected region of the malarial genome. This is a specific and sensitive method and permits genotyping. Drug-resistant parasites and mixed infections can be detected by PCR using single nucleotide polymorphism. A PCR-based detection of *Plasmodium falciparum* in human urine and saliva samples has been described. The antibodies against asexual blood stages of malaria parasite can be detected by the immunofluorescence assay (IFA). Serological tests are useful in epidemiology surveys and are not suitable for the acute diagnosis of malaria [32].

1.2.4 Pulmonary Babesiosis

Babesiosis is caused by hemoprotozoan parasites, *Babesia microti* and *Babesia divergens* [33]. Man gets the infection by the bite of an infected tick, *Ixodes scapularis*, and can also be infected from a contaminated blood transfusion [34]. The parasites attack the red blood cells and can be misdiagnosed as *Plasmodium*. The symptoms are fever, drenching sweats, tiredness, loss of appetite, myalgia, and headache. Acute respiratory distress syndrome occurring a few days after initiation of medical therapy is the important pulmonary manifestation [35]. Chest radiological features include bilateral infiltrates with an alveolar pattern and thickening of the septa. The peripheral blood smears may show, in addition to ring forms, tetrads inside the red blood cells. These tetrads, known as Maltese cross formations, are pathognomonic of babesiosis because they are not seen in malaria [36]. Specific diagnosis is made by examination of a Giemsa-stained thin blood smear, DNA amplification using PCR, or detection of specific antibody [33].

1.2.5 Pulmonary Toxoplasmosis

Toxoplasmosis is caused by one-celled protozoan parasite, *Toxoplasma gondii*. Cats are the primary carriers of the organism [37]. Man gets the infection by eating parasitic cyst-contaminated raw or undercooked meat, vegetables, or milk products. The symptoms of toxoplasmosis are flu-like syndrome, enlarged lymph nodes, or myalgia. Chronic toxoplasmosis can cause chorioretinitis, jaundice, encephalitis, and convulsions. Pulmonary toxoplasmosis has been reported with increasing frequency in patients with HIV infection. Toxoplasma pneumonia can manifest as interstitial pneumonia/diffuse alveolar damage or necrotizing pneumonia [38]. Diagnosis of toxoplasmosis is based on the detection of the protozoa in body tissues. Antibody levels can be increased without active disease. A real-time PCR-based assay in BAL fluid has been reported in immunocompromised HIV-positive patients [39].

1.3 Helminthic Parasites

The important helminthic parasites that cause lung diseases include cestodes (*Echinococcus granulosus* and *Echinococcus multilocularis*), trematodes (*Schistosoma haematobium*, *Schistosoma mansoni*, *Schistosoma japonicum*, and *Paragonimus westermani*), and nematodes (*Ascaris lumbricoides*, *Ancylostoma duodenale*, *Necator americanus*, *Strongyloides stercoralis*, *Wuchereria bancrofti*, *Brugia malayi*, *Brugia timori*, *Dirofilaria immitis*, *Dirofilaria repens*, *Toxocara canis* or *cati*, and *Trichinella spiralis*).

1.3.1 Pulmonary Hydatid Disease

The parasite species that cause hydatid disease in man are *Echinococcus granulosus* and *Echinococcus multilocularis*.

1.3.2 Cystic Hydatid Disease

The adult *E. granulosus* resides in the small intestine of the definitive hosts, mainly dogs. The intermediate hosts including man are infected by ingestion of eggs excreted in the feces of the dogs. Primary pulmonary cystic hydatid disease is usually single. Multiple cysts may be seen in 20 % of cases and may be unilateral or bilateral [2]. Secondary metastatic pulmonary cystic

hydatid disease may occur by the rupture of a liver cyst in vena caval circulation or a heart cyst in the right ventricular cavity. Patients are asymptomatic in the initial stages of infection. Pulmonary symptoms include cough, fever, dyspnea, and chest pain [40]. Signs and symptoms can occur due to compression of adjacent tissue by the cysts. Rupture of the cysts into a bronchus may result in hemoptysis and expectoration of cystic fluid containing parasite membrane and can cause anaphylactic shock, respiratory distress, asthma-like symptoms, persistent pneumonia, and sepsis [41, 42]. Rupture of the cyst into the pleural space can result in hydropneumothorax, pleural effusion, and empyema. Diagnosis of pulmonary hydatid cyst is based on thoracic imaging studies (chest radiography, thoracic computerized tomography (CT), and thoracic ultrasonography) [43] and an uncomplicated cyst presents as a well-defined homogenous round (cannonball) opacity that may be lobulated by contiguous bronchovascular axes (Fig. 1.2) [2]. Chest radiographs show solitary or multiple round opacities that may mimic lung tumors. CT is helpful in doubtful cases, because the internal structure of the cyst can be analyzed and its density measured. CT is also useful to assess the state of the neighboring parenchyma and to evaluate the whole thorax and abdomen for associated cystic lesions or anomalies. Ultrasonography using a portable ultrasound scanner has been found as reliable, inexpensive, and rapid technique in community-based screening surveys for cystic hydatid disease. The crescent sign, Cumbo's sign (onion peel sign), water lily sign, and air-fluid level are seen on chest radiography and computed tomography (CT) [41]. Inverse crescent sign, signet ring sign, and serpent sign are recognized as features of pulmonary hydatid cysts in computerized tomogram.

Laboratory tests are complementary to clinical and imaging investigations. Eosinophilia and elevated immunoglobulin E (IgE) levels are seen when the hydatid cyst ruptures [40]. Serologic tests are less sensitive in patients with lung hydatid disease than in those localized in liver. False-positive tests may be observed in patients suffering from other helminthic infections.

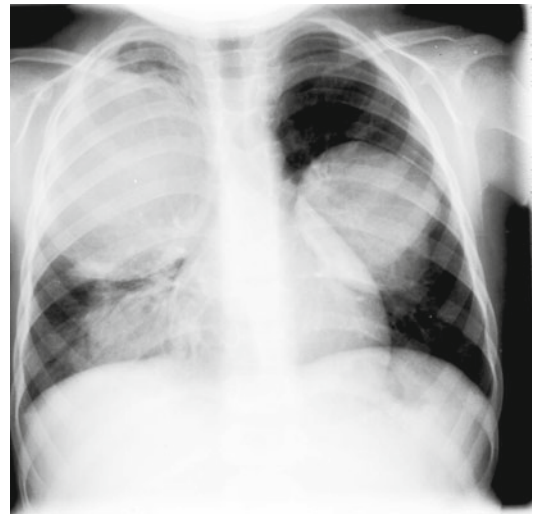

Fig. 1.2 Bilateral typical "cannon ball" images of non-complicated hydatid cysts on chest X-rays (Adapted from Vijayan and Kilnai [2])

Immunologic tests may be helpful to confirm the hydatid origin of a cystic lesion and permit the serologic monitoring of medically or surgically treated patients [44, 45].

1.3.3 Pulmonary Alveolar Echinococcosis

Pulmonary alveolar echinococcosis (AE) is due to hematogenous dissemination from hepatic lesions. The liver is the first target of the parasite, with a silent and long incubation period (5–15 years). Exogenous proliferation causes infiltration of adjacent tissues and pressure necrosis. It can metastasize to distant organs mainly to lungs, brain, and bones [46]. Lung involvement results from metastatic dissemination or direct extension through the diaphragm of hepatic echinococcosis with intrathoracic rupture into the bronchial tree, pleural cavity, or mediastinum. Direct extension to the right atrium through the inferior vena cava with recurrent episodes of pulmonary embolism has also been reported. Imaging studies with radiography, ultrasonography, CT, and magnetic resonance imaging (MRI) may help in the diagnosis of metastatic lung disease [2]. Biopsy may be needed to confirm the diagnosis [47]. Serologic

tests (ELISA, indirect hemagglutination assay [IHA]) are available and are of great value for early detection in endemic areas to confirm the diagnosis and to plan early surgery. Immunodiagnostic tests using purified *E. multilocularis* antigens have good diagnostic sensitivity and specificity for the diagnosis of AE [48].

1.3.4 Pulmonary Schistosomiasis

1.3.4.1 Clinical Diagnosis

The schistosomes that cause human disease are *Schistosoma haematobium*, *Schistosoma mansoni*, and *Schistosoma japonicum*. The final habitat of *S. haematobium* is urinary bladder vesicle beds and of *S. mansoni* and *S. japonicum* is the mesenteric beds. The schistosome eggs are passed in urine (*S. haematobium*) or in feces (*S. mansoni* and *S. japonicum*) by the infected humans. The parasites can cause *Schistosoma* dermatitis at the site of skin penetration. Pulmonary schistosomiasis can manifest clinically as an acute form and a chronic form [49]. Acute symptoms can develop 3–8 weeks after skin penetration [50]. The acute form, also known as Katayama syndrome, presents with fever, chills, weight loss, diarrhea, abdominal pain, myalgia, and urticaria and is seen in nonimmune patients [51]. Pulmonary manifestations include shortness of breath, wheezing and dry cough. Patients with chronic schistosomiasis present with features of pulmonary hypertension and cor pulmonale [52]. Massive hemoptysis and lobar consolidation and collapse have been reported in schistosomiasis [53]. Hepatosplenomegaly due to portal hypertension has been reported in patients infected with *S. mansoni* or *S. japonicum* [49]. Chest radiographic abnormalities range from multiple nodules to diffuse interstitial infiltrates. Small pulmonary nodules in CT have been described in acute schistosomiasis [54].

1.3.4.2 Laboratory Diagnosis

Diagnosis of chronic schistosomiasis is based on the demonstration of eggs in stool or urine by direct microscopy or rectal/bladder biopsy [55]. Multiple examinations of specimens are required in mild and chronic infections. In active infections, eggs contain live and mature miracidia. The incubation period of the infection is usually 3 months and hence eggs can be detected after 3 months of last known contact with fresh water. Peripheral blood eosinophilia with mild leukocytosis, abnormal liver function test results and elevated IgE levels are reported in acute schistosomiasis. Hyperglobulinemia is observed in chronic schistosomiasis. Serological tests with ELISA are available, but cannot differentiate active from past infections [56]. Bronchoscopy and transbronchial biopsy may reveal eosinophilic pneumonitis.

1.3.5 Pulmonary Paragonimiasis (Lung Fluke)

1.3.5.1 Clinical Diagnosis

Paragonimiasis is a food-borne zoonoses and is caused by infection with *Paragonimus* species and manifests as subacute or chronic inflammation of the lung. Though more than 50 species are known to cause paragonimiasis in man, the main species that cause paragonimiasis is *Paragonimus westermani*. Adult worms live in the lungs and the eggs are voided in sputum or feces. The man gets infection, when raw or undercooked crabs or crayfishes infected with infective metacercariae are ingested. The parasite from the human gut passes through several organs and tissues to reach the lung. Pulmonary paragonimiasis manifests as fever, chest pain, chronic cough, and blood-tinged sputum [57]. The cough is dry at first and later productive with blood-stained, rusty-brown tenacious sputum. Occasionally, there is profuse hemoptysis. Pulmonary paragonimiasis is confused with tuberculosis as the symptoms in both diseases are similar. Chest radiographs may show infiltrative, nodular, and cavitating shadows. Pleural effusion or pneumothorax is an important finding in paragonimiasis [58, 59]. CT scan may show single or multiple nodules in the lung parenchyma, calcified spots and pleural thickening with interlobar pleuritis, and pleural effusion. MRI may show conglomerated lesions with hemorrhage or tunnel signs.

1.3.5.2 Laboratory Diagnosis

Definitive diagnosis is based on the demonstration of eggs in sputum samples, BAL fluid, or lung biopsy specimens. Eggs are not present until 2–3 months after infection. Eggs or juvenile forms or adult worms can also be demonstrated in a subcutaneous lump or aspirated pleural effusion. Peripheral blood eosinophilia and elevated serum IgE levels are seen in >80 % of patients with paragonimiasis. A variety of immunological methods including ELISA, Dot immunogold filtration assay (DIGFA), indirect hemagglutination, and indirect fluorescence antibody tests have been used for diagnosis with variable results [2, 60].

1.3.6 Pulmonary Ascariasis

1.3.6.1 Clinical Diagnosis

Ascaris lumbricoides is the most common intestinal helminthic infection. Respiratory symptoms in ascariasis are due to larval pulmonary migration, airway hyper-reactivity, and bronchospasm. Symptomatic pulmonary disease may range from mild cough to Loffler's syndrome [61]. Loffler's syndrome is a self-limiting inflammation of the lungs and is associated with blood and lung eosinophilia. This syndrome can occur as a result of parasitic infestations (especially ascariasis in children) and exposure to various drugs. Patients may present with general symptoms of malaise, loss of appetite, fever lasting 2–3 days, headache, and myalgia. The respiratory symptoms include chest pain, cough with mucoid sputum, hemoptysis, shortness of breath, and wheezing [62]. There may be rapid respiratory rate and rales can be heard on auscultation. Leukocytosis particularly eosinophilia is an important laboratory finding. Chest radiographs demonstrate unilateral or bilateral, transient, migratory, and non-segmental opacities of various sizes. These opacities are often peripherally situated and appear to be pleural based [63]. The severity of symptoms will depend upon the larval burden. Rarely chronic eosinophilic pneumonia or symptoms of upper airway obstruction can occur.

1.3.6.2 Laboratory Diagnosis

A diagnosis of pulmonary disease due to ascariasis can be made in an endemic region in a patient who presents with dyspnea, dry cough, fever, and eosinophilia. Sputum may show Charcot-Leyden crystals and the chest radiograph may reveal fleeting pulmonary infiltrates. Because of the occurrence of respiratory symptoms during larval pulmonary migration, stool examination usually does not show *Ascaris* eggs and stool samples may be negative until 2–3 months after respiratory symptoms occur, unless the patient was previously infected. However, larvae can sometimes be demonstrated in respiratory or gastric secretions [64]. It has been suggested that measurement of *Ascaris*-specific IgG4 by ELISA may be useful in the serodiagnosis of ascariasis [65].

1.3.7 Pulmonary Ancylostomiasis

1.3.7.1 Clinical Diagnosis

Hookworm disease in humans results from infections with two species, *Ancylostoma duodenale* and *Necator americanus*. During pulmonary larval migration, patients may present with fever, cough, wheezing, and transient pulmonary infiltrates in chest radiographs. This is associated with blood and pulmonary eosinophilia [3]. The other characteristic feature is iron deficiency anemia due to chronic blood loss [66]. In severe hookworm anemia, patients may present with fatigue, exertional dyspnea, poor concentration, and cardiac murmurs. During massive infection from oral ingestion of hookworm larvae, patients can present with nausea, vomiting, cough, dyspnea, and eosinophilia, and this condition is termed as Wakana disease. Prominent gastrointestinal symptoms in hookworm disease are abdominal pain, nausea, anorexia, and diarrhea.

1.3.7.2 Laboratory Diagnosis

A direct microscopical examination of stool demonstrates the presence of characteristic hookworm eggs. Concentration method may be used when the infection is light. Eosinophilia in the peripheral blood is a prominent finding. A peripheral blood smear examination will

reveal microcytic hypochromic anemia. A polymerase chain reaction (PCR) to differentiate between *A. duodenale* and *N. americanus* has been developed [67].

1.3.8 Pulmonary Strongyloidiasis

1.3.8.1 Clinical Diagnosis

Strongyloides stercoralis is seen worldwide and the unique feature of the life cycle of *S. stercoralis* is that it can complete its life cycle either in the human host or in the soil. It has been observed that 15–30 % of chronically infected people may be asymptomatic. Although symptoms in individuals with chronic *Strongyloides stercoralis* infection are usually mild, it can persist for many years due to autoinfection. This may occasionally progress to the hyperinfection syndrome with high mortality especially in immunosuppressed individuals [68, 69]. The relative risk of *S. stercoralis* infection is increased in elderly men and patients who had recently used corticosteroids, had a hematologic malignancy, and had prior gastric surgery. Other risk factors include chronic lung disease, use of histamine blockers, or chronic debilitating illness. Strongyloidiasis is a chronic relapsing illness of mild to moderate severity characterized by gastrointestinal complaints (diarrhea, pain, tenderness, nausea, vomiting), peripheral blood eosinophilia, and hypoalbuminemia.

Pulmonary signs and symptoms include cough, shortness of breath, wheezing, and hemoptysis. In patients at high risk for strongyloidiasis, adult respiratory distress syndrome and septicemia due to intestinal transmural migration of bacteria can occur as a result of hyperinfection or disseminated strongyloidiasis [70, 71]. In addition, acute anemia, acute renal failure, and systemic inflammatory response syndrome are also reported in hyperinfection. Strongyloidiasis can manifest as eosinophilic pleural effusion in both immunocompetent and immunocompromised individuals. Rare pulmonary manifestations include acute respiratory failure due to respiratory muscle paralysis, granulomatous reaction in the lung with interlobular septal fibrosis, and pulmonary microcalcifications. A paradoxic therapeutic response of asthma to glucocorticosteroids, in which bronchial asthma symptoms worsened after treatment with parenteral corticosteroids, has been described in patients with strongyloides superinfections [72]. Exacerbations of chronic obstructive pulmonary disease and worsening of symptoms in idiopathic pulmonary fibrosis have also been reported in *Strongyloides stercoralis* infection.

1.3.8.2 Laboratory Diagnosis

In immunocompetent patients with strongyloidiasis, the parasite load is usually low and the larval output is irregular. As a result, the diagnosis of strongyloidiasis by examination of a single stool specimen using conventional techniques usually fails to detect larvae in up to 70 % of cases [73]. The diagnostic yield can be increased by examination of several stool specimens on consecutive days. Examination of stool by agar plate culture method was found to be superior to direct smear and modified Baermann technique [74, 75]. *Strongyloides stercoralis* larvae can be demonstrated in duodenal aspirate. In disseminated disease, larvae and adult parasites can be seen in sputum, urine, bronchoalveolar lavage fluid, and other body fluids [76]. A serological test using Centers for Disease Control (CDC) enzyme immunoassay (EIA) for detection of antibodies to strongyloidiasis was found to have a sensitivity of 94.6 % in patients with proven infection [77].

1.3.9 Tropical Pulmonary Eosinophilia

1.3.9.1 Clinical Diagnosis

Tropical pulmonary eosinophilia (TPE) results from immunologic hyper-responsiveness to human filarial parasites, *Wuchereria bancrofti* and *Brugia malayi* [78–80]. TPE is a systemic disease involving mainly the lungs, but other organs such as liver, spleen, lymph nodes, brain, and gastrointestinal tract may also be involved. The disease occurs predominantly in males, with a male to female ratio of 4:1, and is mainly seen in older children and young adults between the

ages 15 and 40 years. The systemic symptoms include fever, weight loss, and fatigue. Patients with TPE usually present with respiratory symptoms that include paroxysmal cough, breathlessness, and wheeze and chest pain [81, 82]. The symptoms occur predominantly at night, but can persist during the day. Severe cough can lead to fractured ribs. Sputum is usually scanty, viscous, and mucoid. The sputum often shows clumps of eosinophils, and rarely Charcot-Leyden crystals are observed. On examination, patients are often breathless. Bilateral scattered rhonchi and rales may be heard on auscultation [61, 78].

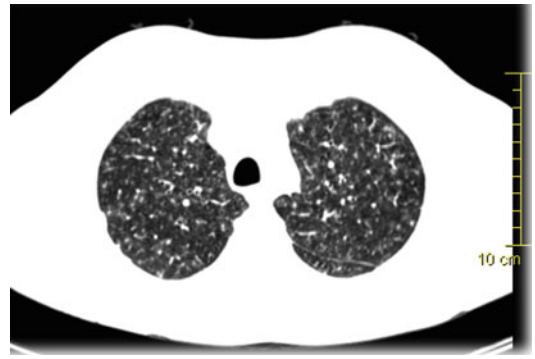

Fig. 1.3 HRCT scan of a patient with TPE showing bilateral nodular shadows (Adapted from Vijayan and Kilnai [2])

1.3.9.2 Laboratory Diagnosis

Leukocytosis with an absolute increase in eosinophils in the peripheral blood is the hallmark of TPE. Spontaneous fluctuations in the eosinophil count can occur. Absolute eosinophil counts are usually more than 3,000 cells/mm^3 and may range from 5,000 to 80,000 [83]. Microfilariae are rarely seen in the peripheral blood. As patients with TPE especially from endemic areas can be simultaneously infected with other helminthic parasites, stool examination may reveal ova or larvae of other helminthes (*Ascaris*, *Ancylostoma*, whipworm, and *Strongyloides*) in 20 % of patients with TPE. This observation does not deter the physician from making a diagnosis of TPE, if other conditions for diagnosis are fulfilled.

The chest radiological features of TPE include reticulonodular shadows predominantly seen in mid and lower zones and miliary mottling of 1–3 mm in diameter often indistinguishable from miliary tuberculosis (Fig. 1.3). Twenty percent of patients have a normal chest radiograph. In patients with a long-standing history, a few patients have honeycomb lungs. Radiological improvement occurs on specific therapy with DEC, but some degree of radiological abnormality persists in some patients. Lung function tests reveal mainly a restrictive ventilation defect with superimposed airway obstruction [84, 85]. Single breath carbon monoxide transfer factor (TLCO) is reduced in 88 % of untreated patients with TPE. The reduction in TLCO is due to reduced pulmonary membrane diffusing capacity (Dm) [86]. The criteria suggested for the diagnosis of TPE are (a) appropriate exposure history (mosquito bite) in an endemic area of filariasis, (b) a history of paroxysmal nocturnal cough and breathlessness, (c) chest radiographic evidence of pulmonary infiltrations, (d) leukocytosis in blood, (e) peripheral blood eosinophils more than 3,000 cells per cu mm, (f) elevated serum IgE levels, (g) elevated serum antifilarial antibodies (IgG and/or IgE), and (h) a clinical response to diethylcarbamazine citrate [87, 88].

1.3.10 Pulmonary Dirofilariasis

Pulmonary dirofilariasis is a zoonotic infection caused by filarial nematodes, *Dirofilaria immitis* and *Dirofilaria repens*. Humans are accidental hosts of this parasite which is transmitted to man by the mosquito. The parasites are usually seen in the pulmonary artery where they produce an embolism ultimately leading to the formation of a pulmonary nodule or "coin lesion" [89]. Nearly 50 % of subjects infected with dirofilariasis are asymptomatic. Clinical symptoms are chest pain, cough, fever, hemoptysis, and dyspnea. CT scan may show a well-defined nodule with smooth margin connected to an arterial branch [90]. Positron emission tomography scan can demonstrate hypermetabolic activity in a pulmonary infarct secondary to dirofilariasis [91]. A PCR-based diagnosis of *D. repens* in human pulmonary dirofilariasis has been reported [92]. A definitive histopathological diagnosis of pulmonary dirofilariasis can be made

in tissue specimens obtained by wedge biopsy, by video-assisted thoracoscopy, or rarely by fine needle biopsy.

1.3.11 Visceral Larva Migrans

Toxocara larva migrans syndromes are important zoonotic infections. Certain nematode parasites entering into an unnatural host (e.g., man) may not be able to complete their life cycle and their progress is arrested in the "unnatural host." The common parasites that cause visceral larva migrans (VLM) and eosinophilic lung disease in man are a dog ascarid (*Toxocara canis*) and less commonly a cat ascarid (*Toxocara cati*) [93]. Human toxocariasis occurs in all parts of the world wherever there is a large pool of infected dogs.

Visceral larva migrans (VLM) is characterized by leukocytosis and eosinophilia. The larva induces a granulomatous reaction in the tissues containing eosinophils and multinucleated giant cells. Larvae can get encapsulated within the granuloma where they are either destroyed or persist for many years in a viable state. Granulomata are found in the lungs, liver, central nervous system, and eyes. Later fibrosis and calcification occur. Larval antigens can cross-react with human A and B blood group antigens.

1.3.11.1 Clinical Diagnosis

Visceral larva migrans is usually reported in young children with a history of pica. A history of exposure to puppies or dogs supports the diagnosis of VLM. These children usually present with fever, cough, wheezing, eosinophilia, and hepatomegaly. However, most of the children infected with *Toxocara* spp. are asymptomatic. The main symptoms in patients with visceral larva migrans are fever, cough, wheezing, seizures, anemia, and fatigue. Pulmonary manifestations are reported in 80 % of cases and patients may present with severe asthma [94]. Scattered rales and rhonchi are heard on auscultation. There will be intense blood eosinophilia. Skiagram chest may reveal focal patchy infiltrates. In some cases, severe eosinophilic pneumonia may lead to respiratory distress [95]. Other clinical features include generalized lymph node enlargement, hepatomegaly, and splenomegaly.

1.3.11.2 Laboratory Diagnosis

Skiagram chest may show patchy infiltrates. Nonspecific changes include hypergammaglobulinemia and elevated isohemagglutinin titers to A and B blood group antigens. Serological tests by ELISA method using excretory-secretary proteins obtained from cultured *T. canis* may be useful in the diagnosis. Cross reactivity with other helminths limits the usefulness of this test in endemic areas. Detection of IgE antibodies by ELISA and toxocara excretory-secretary antigens by Western blotting procedure have also been reported for diagnosis [96, 97]. However, serodiagnostic procedures cannot distinguish between past and present infections. Histopathological examination of lung or liver biopsy specimens may demonstrate granulomas with eosinophils, multinucleated giant cells, and fibrosis. Since man is not the definitive host of *Toxocara* sp., eggs or larvae cannot be demonstrated in the feces.

1.3.12 Pulmonary Trichinellosis

Human trichinellosis is an important food-borne zoonosis. The most important species that infect man is *Trichinella spiralis*. The parasite has a direct life cycle with complete development in one host (pig, rat, or man). Man gets infection from raw and partially cooked pork, when infected pig's muscle containing larval trichinellae is eaten by man. The common symptoms of trichinellosis are muscle pain, periorbital edema, fever, and diarrhea [98]. Pulmonary symptoms include dyspnea, cough, and pulmonary infiltrates. Dyspnea may be due to the involvement of diaphragm [99]. Leukocytosis, eosinophilia, and elevated levels of serum muscle enzymes (creatine phosphokinase, lactate dehydrogenase, aldolase, and amino transferase) are important laboratory findings. An enzyme-linked immunosorbent assay (ELISA) for detection of anti-Trichinella antibodies using excretory-secretary antigens may be useful in the diagnosis. A definitive diagnosis can be made by muscle biopsy

(usually deltoid muscle) that may demonstrate larvae of *T. spiralis* [99].

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Molecular Pathology of Pulmonary Parasitic Diseases

2

Juan P. Olano

2.1 Parasites

In this chapter, I will explore the advances made in understanding the molecular pathogenesis and basic immunological principles of the most common protozoan and metazoan organisms that affect the lungs. Needless to say, these eukaryotic organisms are far more complex genetically than their bacterial and viral counterparts. Genome sizes range from 7,000 to 20,000 protein-encoding genes [1]. During the past 10 years, genome sequences of helminthes and protozoans have been completed or are underway. To date more than 1,000,000 ESTs (expressed sequence tags) are available at GenBank (excluding *Caenorhabditis elegans*) [2]. This level of complexity is needed in order to survive through multiple stages of development that occur in intermediate and definitive hosts. As a rule, most parasitic diseases lead to chronicity, suggesting that the host-parasite relationship enters a level of "tolerance" that we are beginning to understand at the molecular level through a complex interaction between parasite-derived immunomodulatory products and the host immune response.

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2.2 Principles of Parasitic Molecular Pathogenesis

The term pathogenesis encompasses several steps including transmission, entry, initial spread from point of entry to other organs, contact with target cell/organ, survival within the host (immune evasion and adaptation to the host environment), and extension of the niche (multiplication, survival, and modulation of host biology).

Mechanisms of transmission and entry of pulmonary parasites are as diverse as the parasites capable of causing pulmonary disease (see mode of transmission under each pathogen heading). Most of the discussion in this section will focus on the interaction between the parasite and the host, with special emphasis on the immune response as a pathogenetic mechanism in some of these infections.

Human parasitic infections range from asymptomatic carriers to lethal infections. This variability is determined by several factors including host response, virulence factors, and infectious dose. From the immunological point of view, responses to parasites follow the usual Th1 and Th2 types [3]. Th1 responses are associated with the activation of cells by IFN-γ(gamma) and IL-2, induction of cytolytic CD8+ T-cells, and production of complement-fixing antibodies. Th1 responses are very useful when the host is battling an intracellular infectious agent [4]. Th2 responses are driven by IL-4,

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IL-5, IL-10, and IL-13 and are characterized by high levels of neutralizing antibodies and cell-bound antibodies, activation of mast cells, and eosinophils. Th2 responses are most useful for extracellular organisms. Both responses can become detrimental to the host if unchecked due to side effects produced by their effectors such as NO, ROS, and TNF-α(alpha) in Th1 responses and formation of immune complexes, complement activation, and hypersensitivity reactions in strong Th2 responses. As a rule, the host tries to strike a balance between these two responses. Inhibitors of the Th1 response include IL-10, TGF-β(beta), and IL-4; the Th2 response is mostly controlled by IFN-γ(gamma), IL-12, and IL-10. Experimental rodent models have shown that IL-10-deficient mice have increased mortality with normally avirulent *Toxoplasma gondii* (an intracellular protozoan) due mostly to overproduction of IFN-γ(gamma), TNF-α, and IL-12 [5–7]. At the same time, *T. gondii* levels in these animals were lower than their control counterparts. On the other hand, Th2 responses can also be beneficial and detrimental. It has been widely believed that *Schistosoma* spp. cause disease due to the granulomatous response to egg deposition in tissues, especially in the mesenteric circulation and liver leading to scarring and subsequent portal hypertension. In these cases, the host develops a strong Th2 response driven mostly by IL-4 leading to granuloma formation around the eggs. However, in animal models where the IL-4 response is nonexistent, and therefore granuloma formation is markedly impaired, the host succumbs to acute infection rapidly, for the most part due to the deleterious effects of a sustained proinflammatory cytokine response [8–10]. Even though these animal models illustrate the protective role of the Th2 response during the early phases of the infection, the sequelae in the liver during chronic infections, namely, hepatic fibrosis, seem to be driven by IL-13, a potent fibrogenic Th2 cytokine [11]. It appears then that in these animal models, polarized Th1 and Th2 responses are detrimental to the host, whereas a balanced Th1/Th2 response is necessary for the control of egg-induced immunopathology [10, 12].

2.2.1 Innate Immunity to Parasitic Infections

The traditional view of innate immunity playing a small role in parasitic infections has changed in recent years. Innate immunity plays a very important role in determining the class of the adaptive immune response, be it Th1 or Th2 dominated. Innate immunity operates through both humoral and cellular mechanisms. One of the best-studied humoral mechanisms is the activation of the complement cascade through the alternative pathway (parasite membrane components) or lectins present on the parasite surface. Activation of the classical pathway requires parasite Ag/Ab complexes and therefore occurs when adaptive immunity is already active and production of antibodies is underway. Parasites have developed different strategies present at certain developmental stages that prevent killing by the activated complement cascade and include expression of parasite-derived regulatory proteins (gp160 or gp63) or acquisition of regulatory proteins from the host on the parasite surface such as decay accelerating factor (DAF) and factors H and I which inhibit formation of the membrane attack complex (MAC) by acting on C3b [13]. The glycoprotein gp160 present in trypomastigotes of *Trypanosoma cruzi* is homologous to DAF and therefore binds to C3b or C4b, inhibiting the downstream members of the cascade. On the other hand, gp63 present on *Leishmania* spp. can cleave C3b to its inactive from, iC3b, and prevent deposition of the C5b-C9 attack complex [13]. Other examples include the cleavage of C3 by a cysteine proteinase from *Entamoeba histolytica* that leads to complement activation via the alternative pathway, but it also inactivates C3a and C5a, preventing immunoregulatory and chemotactic functions of these two molecules [14]. Amebas and certain helminths like *Echinococcus granulosus* can also acquire host regulatory proteins on their surface [14, 15]. Larvae and adults of *S. mansoni* can also express DAF on their tegumental surfaces [15].

Examples of evasion of innate cellular mechanisms include the ability of *Leishmania* spp. to survive in the macrophage after phagocytosis due

to internalization via complement receptors CR1 and CR3, which fails to trigger the respiratory burst [16]. *T. cruzi* escapes the phagosome by expressing a C9 homolog that disrupts the phagosomal membrane [17]. *T. gondii* prevents acidification of the parasitophorous vacuole [18]. The host at the same time responds to intracellular pathogens by producing IL-12, which activates NK cells and macrophages to control the intracellular pathogen. IL-12 is produced by several cells including macrophages, dendritic cells, and neutrophils. The most studied molecule in protozoans that is thought to be responsible for the production of proinflammatory cytokines is the lipid molecule known as glycosylphosphatidylinositol (GPI) [19, 20]. On the other hand, LPG and GIPLS of *Leishmania* spp. have been shown to downregulate production of IL-12 and TNF-R, therefore favoring the parasite in its initial interaction with the host [21, 22].

NK cells seem to play a very important role in the initial response to parasitic infections. The early production of IFN-γ by these cells seems to prevent the parasite from rapid proliferation in the host [23]. Ultimately, adaptive immunity takes over, and T-cells are the main players in controlling intracellular protozoan infections.

Regarding helminths, innate humoral mechanisms are associated with resistance to such infections. T-cell-dependent mast cell responses play a role in infections caused by nematodes. Eosinophils are also important in resistance to helminthic infections during their larval stages, which requires IL-5 production by T-cells and opsonization (antibody production). Other components of the innate immune response that have been shown to play a role in protection against parasites include B1 cells (B-cells that express the CD5 molecule and are mostly present in body cavities) and γ(gamma)δ(delta)-T-cells which seem to play an important role in epithelial mucosal barriers [23].

2.2.2 Adaptive Immune Response

The ultimate effector mechanisms that control parasitic infections depend largely, like with any other infectious agents, on location within the host (organ and intracellular versus extracellular), life cycle stages within the host, and evasion strategies of the parasite. IL-12 plays a critical role in starting protective immune responses against intracellular parasites. IL-12 upregulates production of IFN-γ and therefore favors Th1 responses [24]. The main sources of IL-12 are macrophages and dendritic cells which secrete IL-12 after ingestion of whole parasites or parasite products. Other sources include T-cells through ligation of CD40 by CD40 ligand and ligation of CCR5 on dendritic cells by MIP1a and MIP1b ligands [4, 25, 26]. Killing of parasites present in macrophages is achieved mostly through activation of these cells by IFN-γ(gamma) and TNF-α(alpha). Activated macrophages then produce both reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI). In protozoan infections that target other cells different than macrophages, the immune system has to eliminate the parasites from non-phagocytic cells. CD8+ T-cells seem to play a critical role in this situation since they recognize antigen in the context of MHC class I molecules which are expressed by all cells in the body. In these cases, CD8+ T-cells aid macrophages by producing IFN-γ(gamma) [4]. Humoral immunity also has the potential of playing a role in infections produced by intracellular parasites. Antibodies act in different ways by lysing the pathogen directly, playing a role in opsonization, promoting antibody-dependent cell-mediated cytotoxicity, and blocking invasion [4].

Th2 responses are important in infections produced by intestinal nematodes. Studies rely on animal models, especially rodents, and have revealed that the two most important cytokines are IL-4 and IL-13 in the process of expulsion of intestinal nematodes from the host [27]. Recently, IL-9 has been implicated and seems to play also an important role in the expulsion process [27]. However, the effector mechanisms responsible for the parasite clearance are not clear. The role of eosinophils and antibodies seems nil or absent. In some animal models of helminth infection, mast cells present in the intestinal mucosa may

play an important effector role in clearance of intestinal nematodes by production of specific proteases [28]. Other studies have suggested the role of subtle chemical changes in goblet cells in the intestinal mucosa making the microenvironment for worm survival less amenable [28]. Another important observation is the effect of type 1 responses. Both IL-12 and IL-18 suppress or downregulate the type 2 response leading to delayed expulsion of intestinal nematodes. Most certainly, other factors are in play such as host nutrition. Gut nematodes can also produce immunomodulatory molecules that would tip the balance towards a type 1 response by an IFN-γ(gamma) mimic in *Trichuris muris* infections [29]. Other observations have implicated the size of the initial inoculum as an important determinant of susceptibility or resistance. In cases where the host is infected by a low-level inoculum, susceptibility develops, whereas a large inoculum is associated with a very strong type 2 response followed by expulsion from the host [4].

After successful entry into a host, bacterial and viral infections are usually followed by rapid replication that leads to tissue damage and therefore disease. The outcome is either recovery if the immune system is able to control the infectious burden or the host's demise if the bacterial/viral burden is overwhelming to the host. In some cases, chronic infections arise, in which the host tolerates a certain amount of infectious burden. For parasitic infections, most of these principles apply to protozoan pathogens. In humans, many protozoan infections are followed by a rapid expansion of the infectious agent within the host followed by chronic infection. Chronicity is due to either antigenic variation or the parasite becoming quiescent or dormant. In helminthic infections, expansion of the initial infectious burden is much more complex due to the life cycle of most helminths. For most helminths, adults mate and try to produce eggs or larvae capable of infecting new hosts. In most cases of human infection, these stages are the ones responsible for the pathology observed in human hosts.

An interesting working model for the understanding of immunity in parasitic infections has been proposed [30–32]. The model proposes a 2D immunological map with an inflammatory-regulatory axis and a type 1 and type 2 axis. Four quadrants would result with four possible immune profiles: type 1 inflammatory (autoimmunity and acute bacterial infections), type 1 regulatory (chronic protozoan and mycobacterial infections), type 2 inflammatory (chronic allergies and fibrosis), and type 2 regulatory (helminth infections). The central elements of type 1 and type 2 inflammatory and regulatory responses are Th1, Th2, Th17, and Treg cells, respectively. Each of these cells is driven by signals from the innate branch of the immune system, and they recruit a characteristic set of effector cells (PMNs, alternatively activated macrophages, eosinophils, mast cells, and basophils). Furthermore, Th1, Th2, and Th17 negatively regulate each other, and Treg cells suppress all the other three subsets.

Another level of control of the immune response during protozoan and helminth infections exists in the form of negative costimulators such as cytotoxic T-lymphocyte antigen 4 (CTLA-4 also known as CD152), programmed death-1 (PD-1 or CD279), and B- and T-lymphocyte attenuator (BTLA or CD272). Blockade of these substances would lead to an enhanced immune response and decreased parasitemia, but potential immunopathology in tissues is affected. On the other hand, activation of the negative costimulatory pathways would lead to a decreased immune response, increased parasitemia, and decreased immunopathology [33].

2.2.3 Parasitic Proteases and Their Role in Pathogenesis

A common theme in protozoan and helminthic infections is the presence of proteases which play an important role in virulence and pathogenesis (Tables 2.1 and 2.2). Their roles have been described in establishing, maintaining, and expanding or exacerbating infections [34–36]. Larval stages of several helminth nematodes directly invade the human host through the skin (*Schistosoma*, *Strongyloides*, and *Ancylostoma*)

Table 2.1 Summary of representative parasitic proteases, protease inhibitors, and immunomodulators identified in protozoan pulmonary pathogens

Protease	Parasite	Mechanism(s) of action
EhCP1	*E. histolytica*	Inflammatory dysregulation
EhCP2	*E. histolytica*	Cytotoxic. Disruption of intestinal epithelium Enhancement of chemokine activity (CXCL8)
EhCP5	*E. histolytica*	Enhancement of cytokine activity (IL-1β) Inactivation of IL-18 Caspase 3 activator (apoptotic signal)
Toxopain 1	*T. gondii*	Cysteine protease. Rhoptry biogenesis, protein processing
TgSUB1	*T. gondii*	Serine protease. Host cell invasion
Microneme processing proteases (TgMPP1-3)	*T. gondii*	Host cell invasion
Toxomepsin II	*T. gondii*	Aspartyl protease. Host cell invasion
ROP16 (rhoptry kinase)	*T. gondii*	Down-modulation of IL-12
Microneme protein protease 1 (MPP1)	*T. gondii*	Host cell invasion
Formin/profilin (TgPRF)	*T. gondii*	Host cell invasion, gliding motility, and exocytosis
Myosin motors and associated proteins (TgMyoA, TgGAP45, and TgGAP50)	*T. gondii*	Parasite motility and invasion
TgMIC1/MIC4/MIC6	*T. gondii*	Attachment, internalization
CAD98424	*C. parvum*	Aspartyl protease. Host cell invasion

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Table 2.2 Summary of representative parasitic proteases and protease inhibitors identified in helminth pulmonary pathogens

Protease	Parasite	Mechanism(s) of action
Elastase-like serine proteases	*S. mansoni*	Immunoglobulin degradation
900 kDa glycoprotein ECF-SjE	*S. japonicum*	Eosinophil chemotaxis
440 kDa JAE-H/JAE-L glycoproteins	*S. japonicum*	Eosinophil and neutrophil chemotaxis
Surface glycans	*S. mansoni*	Decreased phagocytosis
Schistosome secreted proteins alpha-1 and omega-1	*Schistosoma* spp.	IL-4 release, basophil degranulation. Favorable Th2 environment
Phosphatidylserine (PS)	*Schistosoma* spp.	DC polarization (IL-4, IL-10 production)
Lyso-phosphatidylserine (lyso-PS)	*Schistosoma* spp.	Increased IL-10 production from Treg cells
Onchocystatin	*O. volvulus*	Inhibition of host cysteine proteases Decreased antigen presentation by APCs and T-cell hyporeactivity Cuticle molting
Bm-CPI-1	*B. malayi*	Inhibition of host cysteine proteases. Unknown
Bm-CPI-2	*B. malayi*	Inhibition of host cysteine proteases (cathepsins L, S, AM) T-cell hyporeactivity via altered MHC antigen presentation
Bm-SPN-1	*B. malayi*	Inhibition of host serine proteases. Regulation of proteolysis
Bm-SPN-2	*B. malayi*	Unknown
SMpi56	*S. mansoni*	Inhibition of host serine proteases Possible inhibition of coagulation cascade and complement activation Inhibition of host neutrophil elastase

(continued)

Table 2.2 (continued)

Protease	Parasite	Mechanism(s) of action
MIF homologue	*B. malayi*	Synergy with IL-4 to increase alternatively activated macrophages (AAMs)
SHSPI	*S. haematobium*	Inhibition of host serine proteases Same as SMpi56
Excreted-secreted proteins (ESPs)	*Paragonimus* spp.	Cysteine proteases. Tissue degradation
Strongylastacin	*S. stercolaris*	Metalloproteinase. Skin invasion
Calreticulin	*N. americanus*	C1q binding, procoagulant sequestration
Macrophage migration inhibitory factor (MIF)	*T. spiralis*	Regulation of host macrophage responses
Glycosphingolipids	*Echinococcus*	Decreases macrophage activation
Glycans	*Echinococcus*	Decreased PMN chemotaxis, modulation of dendritic cells Increased production of IL-4 and IL-13

Source: Reprinted from Olano [120], with permission from Springer Science + Business Media

[37]. The third stage of larval maturation is the invasive one, and several serine and metalloproteases have been found to be expressed during this stage of development. Likewise *Onchocerca* migrates extensively within the body once the infection is established after a vector bite [38]. Tissue migration is indeed mediated by proteases expressed in the mature microfilaria. Trematodes such as *Fasciola* spp., *Paragonimus* spp., and *Clonorchis* spp. also produce several proteolytic enzymes during the tissue invasive stage of the life cycle in order to form a niche in their target organs [39, 40]. In addition, proteases can also play a role in the immunomodulatory process by degrading immunoglobulins or altering cytokine production, especially IL-8 [41]. As a general rule, proteases produced during the larval stage play an important role in tissue invasion or immune evasion, whereas proteases produced during the adult stage primarily degrade gut proteins (those who use the bowel as niche for adult stages) and have roles in anticoagulation and immune evasion.

The best-studied proteases are the ones produced by schistosomes and have been described in several stages of development [42]. They can be present in the parasite gut and excreted or present on the surface of the parasite where they can coat it and play roles in anticoagulation or degradation of immunoglobulins. Some of the proteases are also potent immunogens and could be used as vaccine candidates [43].

Another kind of proteins produced by helminths is protease inhibitors that play an important role in perpetuating these infections for the entire life span of the host. Molecules released from filarial parasites such as phosphorylcholine can interfere with activation and proliferation of T- and B-cells and favor T-helper responses towards the Th2 type. Inhibitors of cysteine and serine proteases, known as cystatins and serpins, respectively, can also have a role in immunomodulation [43].

The best-known cystatin is the one found in *Onchocerca volvulus* (onchocystatin) and is responsible for inhibition of protease activity in antigen-presenting cells (APCs) leading to low T-cell responses [44]. Other cystatins have been described in *Brugia malayi* which inhibit host cathepsins leading to altered digestion of antigens necessary for presentation of antigen-derived peptides to immune cells via MHC molecules [45]. Serpins have been described in *Schistosoma* spp. and are possibly responsible for anticoagulation. *Ascaris* spp., and *Ancylostoma* spp., secrete protease inhibitors that might be responsible for inhibition of host enzymes such as trypsin and chymotrypsin in the gut lumen and inhibition of key coagulation factors [46].

Protozoans also produce potent proteases that play an important role in pathogenesis and immune response. For example, the genome of *E. histolytica* is thought to contain up to 20

proteases, three of which are very well studied and they all belong to the family of cysteine proteases: EhCP1, EhCP2, and EhCP5. Two of them, 1 and 5, are actually absent in other nonpathogenic amebas [47]. They are cytolytic and therefore contribute to intestinal layer degradation and trigger strong inflammatory responses via enhanced activity of chemokines such as CXCL8. Other protozoan proteases have been described in *T. gondii* and their role elucidated during entrance and exit from parasitized cells. Examples include cell surface proteins such as Tg MIC (*Toxoplasma* microneme protein) and TgAMA1 (*T. gondii* apical membrane antigen); proteins present in the rhoptries such as Tg toxopain I and Tg SUB1 and 2, important in processing and targeting rhoptry proteins ROP2, 3, and 4; and proteins of the aspartyl protease group such as toxomepsin II, are also important for invasion [43].

2.3 Molecular Pathogenesis of Pulmonary Protozoan Pathogens

2.3.1 Toxoplasmosis

Toxoplasmosis is a systemic disease due to an obligate intracellular coccidian named *Toxoplasma gondii* which is a very homogeneous species with only three strains worldwide, responsible for more than 95 % of infections. Its distribution is worldwide and the range of animal species that can be affected by it is broad [48]. The life cycle involves a wide range of mammalian intermediate hosts [48]. *T. gondii* is found in three forms in nature, namely, tachyzoites (asexual forms), tissue cysts enclosing bradyzoites (found mostly in the brain and muscle), and oocysts containing sporozoites (sexual forms). The invasive form in humans and other hosts is the tachyzoite and is also the form responsible for cellular and tissue damage. Tissue cysts containing bradyzoites serve as reservoirs for tachyzoites and therefore play an important role in disease transmission (ingestion of contaminated meat) and latent infection. Oocysts with sporozoites are only found in the definitive hosts, the wild and domestic *Felidae*, where they are produced in the intestinal epithelium and then shed in the stool. After sporulation, the cysts are infectious and can then be ingested/inhaled by humans. Other routes of transmission include ingestion of raw or undercooked infected meats, perinatal exposure from infected mothers, transfusion of infected blood products, and transplantation of infected organs. Regardless of the route of infection, tissue cysts or oocysts are digested in the GI tract, and bradyzoites (tissue cysts) or sporozoites (oocysts) are released in the intestine where they invade neighboring cells and become tachyzoites. Invasion of the eukaryotic host cell is an active process mediated completely by the parasite's cytoskeleton [49, 50]. The host cell cytoskeleton does not play any role nor does phosphorylation of any proteins upon attachment of the parasite to the host cell via glycosaminoglycans and sialic acid [51]. The ubiquity of the receptor explains the wide distribution of the parasite in human infections. The parasite adhesins also play an important role in entry. They are not displayed continuously on the surface, as opposed to bacterial and viral adhesins. Instead, the adhesins are contained in cytoplasmic structures called micronemes that discharge their contents upon contact mediated by a controlled release of calcium from the parasite [51]. A "baseline" secretion of the adhesin is sufficient for the initial interaction followed by a dramatic increase in microneme secretion. Such secretion is only apical facilitating interaction in a polarized way that is necessary for entry. The best characterized of the microneme proteins is MIC2 which belongs to the TRAP (thrombospondin anonymous repeat protein) family of proteins [51, 52]. Type A domains (or Von Willebrand factor-like domains) interact with heparin-like molecules and GAGs and therefore are important as adhesins. After apical secretion, MIC2 is transported to the posterior pole of the parasite via the actin cytoskeleton where it is cleaved and released from the cell surface. MIC2 in turn is tightly related to an accessory protein (M2AP) that is also necessary for upregulation of MIC2 secretion from the micronemes. The cytoplasmic domain of MIC2 in turn binds aldolase in the host cell, and

this complex is able to recruit actin monomers [51]. The actual protein responsible for the gliding motility in *T. gondii* is a class XIV myosin that is present beneath the plasma membrane named TgMyoA [53]. This protein is anchored to the inner membrane complex by accessory proteins, and myosin filaments can propel actin filaments recruited by the aldolase-MIC2 complex and induce motility. Entry also depends on a calcium-regulated secretion of the parasite [54]. However, calcium signals in the host cell do not play a role in entry either. Since entry relies completely on active motility of the parasite, research into this area has been active and has elucidated some molecular mechanisms responsible for the unique "gliding" motility seen in all apicomplexans. *T. gondii* motility is highly predictable and consists of circular gliding (counterclockwise) and helical gliding (clockwise). Once inside the cell, *T. gondii* resides in a modified vacuole that does not fuse with any of the endocytic or exocytic vesicles. Most of the vacuolar membranes come from apical organelles called rhoptries which secrete their contents after entry. The main component identified so far is a transmembrane protein called ROP2 which mediates interactions between the vacuolar membrane and the host cell's mitochondria and endoplasmic reticulum [55]. Another component of the vacuolar membrane is the host's glycosylphosphatidylinositol (GPI)-anchored proteins. The damage during the acute infection is due to cell death of parasitized cells and a vigorous inflammatory reaction which initially is neutrophilic in nature and turns lymphocytic when acquired immunity sets in. Most human hosts control the disease in the acute phase, and critical determinants are IL-12 and IFN-γ(gamma) followed by CD8+ T-cells [56, 57]. Antibodies are also capable of neutralizing or killing circulating tachyzoites [58]. Tissue cysts containing bradyzoites then form and tissue integrity is usually restored completely. In some cases, infection persists in the lymph nodes leading to chronic lymphadenopathy, usually in the cervical region accompanied by mild constitutional symptoms. The spectrum of disease ranges from asymptomatic infections (the most common form) to severe disseminated disease seen mostly in immunocompromised patients. In the lungs, the main presentation is that of a diffuse confluent bronchopneumonia that appears secondary to hematogenous and lymphatic dissemination, followed by shock. In AIDS, pulmonary toxoplasmosis is seen in up to 3 % of cases and CD4+ T-cells are usually below 100 cells/μl.

2.3.2 Amebiasis

Pulmonary amebiasis is due to *Entamoeba histolytica*, a protozoan primarily responsible for colonic infections in humans. Pulmonary infections are the result of complications seen in cases of intestinal amebiasis in which *E. histolytica* becomes systemic after invasion of the colonic mucosa, spreading to the liver, lungs, and other organs. Most of these infections occur in the tropics in developing countries. The life cycle of *E. histolytica* comprises an infective cyst and an invasive trophozoite. Cyst formation appears to be mediated by quorum sensing triggered by a lectin (Gal/GalNAc) on the parasite's surface [59]. Excystation occurs in the intestine and eight trophozoites are produced from each cyst which then invade the colonic mucosa leading to ulcer formation. Killing of host cells occurs only after contact of trophozoites with the host cell, and adhesion is mediated by an amebic adhesin, the Gal/GalNAc lectin, referred to above. The lectin recognizes N- and O-linked oligosaccharides on the cell surface. This lectin also appears to be cytotoxic since monoclonal antibodies directed against certain epitope block cytotoxicity in vitro although adhesion is conserved [59]. Invasion and hematogenous spread activates the immune system, and in cases of amebiasis, both the alternate and classical complement pathways are activated. However, trophozoites are resistant to the C5b-C9 attack complex which is inhibited by the abovementioned lectin [60]. Mechanisms of cell killing by *E. histolytica* are under intense scrutiny and may include dramatic rises in cytoplasmic calcium upon contact leading to cell blebbing and death, apoptosis, and a pore-forming protein isolated from ameba [61]. Amebic cytoplasm also contains collagenases and cysteine proteases

that also play a role in pathogenesis by degrading extracellular matrix and producing cell detachment, respectively [43].

Immunity to amebic infections is usually both humoral (secretory IgA antibodies directed against the surface lectin) and cell mediated in the form of cytokine activation of macrophages and neutrophils which become amebicidal after stimulation by IFN-γ(gamma), IL-12, and TNF-α(alpha) [62–64]. Most infections are acquired via the fecal-oral route, but cases can also be acquired via the anal route in homosexual men. Pleuropulmonary complications of amebiasis are seen in cases on hepatic amebiasis (up to 20 %) or invasive colonic amebiasis (up to 3 %). Multiple forms have been described including a pleuritis that results from the inflammatory reaction in the liver "traveling" via the right dome of the diaphragm, empyema or pulmonary amebic complications (pneumonitis, abscess, or fistulas) secondary to rupture of a hepatic abscess, and hematogenous spread.

2.3.3 Microsporidiosis

Phylum Microsporidia are spore-forming, obligate intracellular protozoans that reside in the intestine, liver, kidneys, brain, and other tissues of wild and domesticated mammals and several other animal species. Eight genera out of more than 144 (containing more than 1,000 species) have been documented as human pathogens, namely, *Encephalitozoon*, *Enterocytozoon*, *Pleistophora*, *Brachiola*, *Nosema*, *Trachipleistophora*, *Vittaforma*, and *Microsporidium* [65]. Of these, *Encephalitozoon hellem*, *E. cuniculi*, and *Enterocytozoon bieneusi* have been documented as the main culprits in pulmonary microsporidiosis. Microsporidia in general are rare pathogens in humans that have received attention due to the increased incidence of infections present in patients with AIDS. In cases of pulmonary microsporidiosis, there usually is intestinal involvement and in many cases systemic involvement. Histologically, the microsporidia are seen as faintly basophilic intracellular round structures in the apical portion of the cell measuring 1–1.5 μm in diameter inside epithelial cells lining the bronchial and bronchiolar epithelium. Microsporidia are eukaryotes with Golgi apparatus, mitochondrial remnants, a double-layered spore structure (exo- and endospore layers), and a typical extrusion apparatus anchored to the anterior end of the spore by a disc [65]. Upon invasion of the host cell, the sporoplasm is extruded through the polar tube, which pierces the phagocytic vacuole, into the cytoplasm of the host cell [66]. Spores gain access to humans via ingestion or inhalation. Once they germinate in the host, the sporoplasm undergoes merogony, in which proliferation occurs (meronts), followed by sporogony, in which the membranes thicken again and form sporoblasts that turn into mature spores and are released from the distended cell and into the environment to complete the cycle. Characterization of the process of extrusion of the sporoplasm is lacking. Early events in the process include rupture of the anterior attachment complex upon host cell attachment and cell penetration. So far three polar proteins have been identified and are known as PTP1, PTP2, and PTP3 [66]. PTP1 is O-mannosylated, a post-translational modification that seems to be necessary for its function [67]. PTP1 represents at least 70 % of the polar tube mass. Furthermore, it has been demonstrated in humans that PTP1 is one of the immunodominant proteins that triggers formation of neutralizing antibodies of the IgG type in humans [68]. The major epitope is indeed the posttranslational carbohydrate modification, namely, O-mannosylation. PTP2 is also immunodominant and PTP3 seems to be involved in sporoblast-to-spore polar tube biogenesis [69]. PTP1 and PTP2 contribute to the high tensile strength of the polar tube via extensive disulfide linkages [69]. Another mechanism of infection is phagocytosis upon attachment of the microscoporidia to the host cell. In these cases, penetration of the host cell by the polar apparatus and extrusion of the spore contents do not occur on the cell surface. Instead, the spore is phagocytosed and some of them extrude their contents using the polar tube, thus escaping the endosomes [70]. The spores that remain in the endosomes at some point fuse with lysosomes

and disappear after 72 h. The phagocytosis route was ten times more effective than the attachment followed by extrusion in an in vitro model using *E. cuniculi*. Adhesion mechanisms have also been studied with *E. intestinalis*, and host cell glycosaminoglycans seem to play an important role in the adhesion process. Another interesting pathogenetic mechanism is manipulation of the host cell cycle. In models using *Encephalitozoon*, it has been shown that levels of cyclin D1 are decreased and cyclin B1 are elevated suggesting that host cells can go into arrest to ensure optimal growth of the parasitophorous vacuole in a non-dividing cell [71].

2.3.4 Cryptosporidiosis

Cryptosporidium was first diagnosed as a human pathogen in 1976 in two immunocompromised patients in whom persistent diarrhea developed. The largest outbreak occurred in Milwaukee in 1984 and involved 400,000 people most of whom recovered completely [72]. However, in immunocompromised patients, the diarrhea persists for weeks or months and is debilitating. Currently the main risk factor is the presence of HIV infection/AIDS. Cryptosporidiosis is endemic in developing countries and is responsible for childhood diarrhea. Respiratory cryptosporidiosis results in cough, dyspnea, fever, and chest pain and is always associated with gastrointestinal symptoms. Histologically, there is tracheitis, bronchitis, and bronchiolitis with mild to moderate mononuclear inflammatory infiltrate in the mucosa and submucosa. The organisms are usually seen in the epithelial surface and rarely in submucosal glands. *Cryptosporidium* belongs to the phylum *Apicomplexa* (some other members of the phylum include *Toxoplasma*, *Plasmodia*, and *Babesia*) and is a monoxenous genus (complete developmental cycle occurs in one host) [73]. Oocysts are ingested by the host and release in the small bowel lumen four sporozoites (infective form). Upon attachment, sporozoites form an intracellular/extracytoplasmic parasitophorous vacuole in which they evolve into trophozoites and then into meronts (schizonts). Schizonts undergo three nuclear divisions and become type I merozoites that invade neighboring cells and develop into type II merozoites or into trophozoites. The merozoites can infect other cells and restart the asexual part of the cycle. Type II meronts can also undergo two nuclear divisions and release 4 type II merozoites that invade other cells and become macro- and microgametocytes which can then form a zygote (sexual reproduction). The zygote ultimately becomes an either thin-walled oocyst (autoinfectious) or a thick-walled cyst shed in feces. There are several species within the genus, and the ones known to infect humans include *C. parvum* (humans and bovines), *C. hominis* (humans), *C. meleagridis* (turkeys and humans), and *C. felis* (cats and humans) [73]. Infectious doses are very low. As few as ten oocysts are capable of starting an infection in humans. In addition, cysts are extremely resistant to chlorination treatments and pass through filters relatively easily. Forms of the disease include endemic childhood diarrhea in developing countries, traveler's diarrhea in visitors to endemic countries, chronic diarrhea in immunosuppressed patients, and diarrhea outbreaks in developed countries.

The pathogenesis of these infections is virtually unknown. Several mechanisms have been proposed such as malabsorption produced by villous inflammation and blunting, prostaglandin secretion at the local levels, cellular damage secondary to IL-8 and TNF-α (alpha) secretion, and substance P release in the microenvironment [74–78]. Entry into the unique compartment (intracellular/extracytoplasmic) requires protein kinase C activation and actin rearrangements in vitro [79]. In cultured biliary epithelial cells, *C. parvum* has been shown to induce apoptosis via Fas/FasL interactions [79]. In fact, *C. parvum* is responsible for cases of ascending cholangitis in immunocompromised patients. Full genomic sequence of *C. parvum* "type II" isolate was finalized in 2004 and revealed in 9.1 Mbp in eight chromosomes, coding for approximately 3,807 proteins [80]. *Cryptosporidia* lack mitochondria and apicoplasts, unlike *Plasmodia* and *Toxoplasma*, making the genomes simpler and smaller. Its metabolic pathways are very efficient

and rely mostly on glycolysis as a source of ATP due to absence of mitochondrial genes. Because of its unique intracellular but extracytoplasmic location, several genes are present for transport of sugars and amino acids into the parasitophorous vacuole. Motility and adhesion seem to be mediated by a family proteins known as thrombospondin-related adhesive proteins or TRAPs. In the adhesion process, an apical complex glycoprotein (CSL) has been shown to play an important role and its receptor is an 85-kDa protein in intestinal epithelial cells.

Cell-mediated immunity is important in controlling infections and the most important cytokine seems to be IFN-γ(gamma) [81]. Humoral response appears to be irrelevant. Elevated levels of IL-15 in the intestinal mucosa seem to correlate with no fecal shedding in human volunteers infected with cryptosporidia [82]. Other cytokines elevated found in Haitian children include IL-8, IL-13, and TNF-α(alpha) [74].

2.4 Molecular Pathogenesis of Pulmonary Helminthic Pathogens

2.4.1 Nematodes

2.4.1.1 Filariasis

These infections can be divided into lymphatic filariasis and zoonotic filariasis. The first one is caused by *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori*. The latter is caused by filarial parasites that usually infect other animal hosts, and humans become accidental hosts.

Lymphatic filariasis is the cause of recurrent lymphadenitis leading to sequelae such as elephantiasis and hydrocele due to lymphatic obstruction. In some individuals infected with *W. bancrofti* or *B. malayi*, a distinct asthma-like syndrome develops, known as tropical pulmonary eosinophilia, in which there is paroxysmal cough and wheezing (typically at night due to nocturnal filarial periodicity), low-grade fever, and weight loss. Typically, the blood reveals severe eosinophilia (>3,000/μl) and elevated IgE levels in serum [83]. If not treated, the condition can develop into restrictive pulmonary disease with interstitial fibrosis. The life cycle [83] of lymphatic filariae is very long and starts with the biting of a patient with circulating microfilariae. Once in the mosquito, they develop into first through third stage larvae in the thoracic muscles which then introduce infective third stage larvae into the human circulation. Larvae migrate to lymphatics where they mature and differentiate into adult females and males which then mate and start releasing microfilariae after 5–10 years. Up to 10,000 parasites can be released per day from pregnant female worms.

Initial immune responses to the third and fourth larval stages (early in human infection) are both proinflammatory Th1 and Th2 type of responses [84]. By the time microfilariae appear in the blood, several years later, there are markedly diminished antigen-specific T-cell responses, especially IFN-γ(gamma) and IL-4 [85]. Mechanisms for immune tolerance are multiple and poorly studied but include genetic predisposition, suppressor T-cells, increased expression of downregulatory molecules such as CTLA-4, and high levels of regulatory cytokines such as IL-10 and TGF-β(beta) [86, 87]. Another potentially important pathogenetic mechanism is the presence of large numbers of the endosymbiont *Wolbachia pipientis* in filarial parasites that probably modulate the parasite's life cycle. Dying parasites probably release large numbers of *Wolbachia* cellular products triggering an inflammatory reaction [88–90].

The best-known zoonotic filariasis is the one caused by *Dirofilaria immitis* [91]. These are all transmitted to humans by an infected arthropod and lead to a solitary (sometimes multiple) "coin lesions" in the lungs that are easily confused with neoplastic processes on chest X-rays. Patients are usually asymptomatic and the diagnosis is made when the lesion is taken out for histologic examination. Peripheral eosinophilia can be seen in blood smears. Pathogenetically, the lesion appears as a reaction around a dead or dying worm. *D. immitis* is mainly a dog pathogen although it can infect other mammals such as cats. This nematode has a similar life cycle as described for the lymphatic filariasis. However,

D. immitis is mostly a vascular pathogen [92]. In humans who are resistant to chronic infections, the microfilariae lodge into pulmonary vessels and subcutaneous vessels, and the immune system destroys the parasite, time at which the histopathology appears.

2.4.1.2 Strongyloidiasis

This disease is caused by *Strongyloides stercolaris*. The genus contains more than 40 named species and only one is capable of completing its cycle in humans [93]. The disease is mostly tropical but endemic foci can be seen in temperate areas. The infection is acquired at the time filariform larvae enter the human body via the skin (usually the lower extremities) and gain access to vascular or lymphatic channels that take them to the lungs where they rupture the capillaries and gain access to alveolar spaces. Location of the adequate host depends on detection of thermal and chemical signals by the larvae using specialized amphidial neurons located in the amphidial channel [94]. A metalloproteinase expressed in the third larval stage, named strongylastacin, is possibly responsible for skin penetration. This protein belongs to the metzincin superfamily of zinc metallo-endopeptidases [95]. From the lungs, they migrate up to the bronchi, trachea, and upper aerodigestive tract where they are swallowed and develop into hermaphroditic adults in the small bowel mucosa. The adults then penetrate the mucosa and release eggs that hatch into rhabditiform larvae. At this point of the cycle, two possible scenarios come into play: (1) larvae are shed in the stool and develop into free living male and female adults which mate and release eggs into the soil, followed by hatching of rhabditiform larvae which then develop into filariform larvae (indirect or heterogonic development), and (2) larvae shed in stool develop directly into filariform larvae (direct development). In addition, a third possible scenario is that of autoinfection in which rhabditiform larvae in the bowel lumen mature into filariform larvae which then can invade the body through bowel mucosa or perianal skin. The latter scenario is the one responsible for the so-called pruritic larva currens syndrome and hyperinfection leading to systemic disease (pneumonitis, colitis, polymicrobial sepsis, and meningitis) [93].

Chronic infections with *S. stercolaris* are as a rule asymptomatic. However, in cases of hyperinfection, dissemination is marked, and in the lungs it can lead to diffuse bronchopneumonia, often with intra-alveolar hemorrhage or abscess formation. These infections are usually mixed with intestinal bacteria carried by the parasites in their cuticles. Rhabditiform larvae, filariform larvae, and even eggs can be seen in tissue sections. The mortality rates for hyperinfected patients approach 90 % and are usually associated with administration of exogenous steroids for conditions such as asthma and COPD. Steroids seem to upregulate metabolism of ecdysteroids (molting hormones) in the parasite, via receptor-mediated uptake of the corticosteroid by the parasites [96, 97]. Eggs and rhabditiform larvae receive molting signals, and the number of filariform larvae increases dramatically. Intestinal populations in these cases approach 100,000 adults, and even if steroids are discontinued, and molting rates are low, the burden of adult worms is so high that population growth cannot be arrested. The described developmental processes might be regulated by a family of transcription factors that control genes in response to fat-soluble hormones such as steroids. A gene homolog of daf-12 present in *Caenorhabditis elegans* has been described in *S. stercolaris* [98]. Such gene plays an important role in the development of *C. elegans*.

Other risk factors include HTLV-1 infections, autoimmune diseases, hematologic malignancies, and solid organ allografts [93]. However, the common denominator in many of these conditions seems to be steroid administration. In HTLV-1 infections, it has been demonstrated that the cytokine profile in humans infected with this retrovirus favors the parasite by way of high levels of IFN-γ(gamma) and TGF-β(beta) leading to decreased levels of IL-4, IL-5, IgE, and IL-13 [99]. Interestingly enough, *S. stercolaris* has also been shown to decrease the period of time to develop acute T-cell lymphoma/leukemia (ATLL) in patients infected with HTLV-1. In such cases, *S. stercolaris* induces a significant expansion of restricted T-cell clones infected with HTLV-1

[100]. In average, the incubation period of ATLL is decreased by 30 years in patients infected with HTLV-1.

Other nematodes include (*Ascaris lumbricoides*), hookworms (*Ancylostoma duodenale*, *Necator americanus*), *Toxocara canis* (visceral larva migrans), and *Trichinella* spp.

A. lumbricoides and hookworms are acquired through the mouth, and once eggs hatch in the intestines, larvae migrate throughout the body including the lungs where they cross from capillaries to airways and migrate up to the upper airways to be ingested and mature into adults in the intestine. The larval stage is capable of producing mechanical damage to tissues in the lungs and hypersensitivity reactions elicited by larval antigens leading to pulmonary and bronchial lesions rich in neutrophils, eosinophils, and macrophages (eosinophilic pneumonitis). Pulmonary signs and symptoms are known clinically as Loeffler's syndrome which tends to be more severe in cases of ascaridiasis. Toxocara infections tend to be as severe as ascaridiasis and can also lead to acute or chronic eosinophilic pneumonitis whose severity also depends on the larval burden. Trichinellosis is also a nematode that can affect several organ systems including the lungs and its presentation is similar to the above-described syndromes.

2.4.2 Trematodes

2.4.2.1 Paragonomiasis

The best-known pathogen in the genus *Paragonimus* is *P. westermani*, although seven more species have been described as human pathogens: *P. westermani* is mostly found in the far east (from India to Japan and the Philippines), *P. heterotremus* in China and southeast Asia, *P. skrjabini* and *P. hueitungensis* from China, *P. miyazakii* from Japan, *P. uterobilateralis* and *P. africanus* from central and western Africa, *P. mexicanus* from Central and South America, and *P. kelicotti* from North America [101]. The life cycles are very similar but the best-well-studied one is *P. westermani*. The cycle [102] starts with ingestion of metacercariae in uncooked crab or crayfish. The metacercariae are then excysted in the stomach and small bowel and migrate through the bowel wall, mesenteric fat, and diaphragm until they reach the pleural cavity and lungs where they mature into hermaphroditic flukes that cross-fertilize. Human tissue surround the parasites with a capsule that then cavitates causing hemoptysis and cough. Adults lay eggs after fertilization which are found in sputum and feces if swallowed. The eggs then embryonate in water and miracidia are released which then penetrate *Thiara* or *Semisulcospira* snails. Once in this intermediate host, miracidia turn into sporocysts, rediae, and then short-tailed cercaria. The infected snails are then ingested by crabs or crayfish and cercariae encyst as metacercariae in gills and muscles of these crustaceans. The spectrum of disease ranges from pleuropulmonary infections due to *P. westermani*, *P. heterotremus*, *P. africanus*, and *P. uterobilateralis* to mostly cutaneous manifestations due to *P. skrjabini*. Adults can live up to 5–10 years in human tissues, and acute disease manifestations can happen any time during this period, but usually acute symptoms occur days after ingestion. They include diarrhea and abdominal pain, followed days later by fever, chest pain, fatigue, urticaria, and cough which can turn productive with rusty-colored sputum [102]. Pathologically, the lesions consist of cavitary lesions when adult flukes appear. Excised lesions reveal adult worms with fibrous cysts and egg-induced granulomas. Bronchiectasis, vasculitic lesions, and consolidation can also occur. Other organs affected include the skin, brain, liver, spleen, and peritoneum.

Paragonimus spp. secrete several biologically active molecules called excretory-secretory products (ESPs), and several of them are cysteine proteases whose role is probably host tissue degradation [102, 103]. In addition, they might play a role in immune modulation. In vitro, microglial cells exposed to low levels of ESPs secrete NO, while at high levels microglial cells die [104]. Likewise, co-incubation of eosinophils with ESPs induces rapid degranulation and elevated levels of granule products such as eosinophil-derived neurotoxin [105]. In addition, ESPs can also induce apoptosis in eosinophils via caspase 3 activation, facilitating survival of parasitic

larvae early in the infectious process [106]. Another important mechanism of survival is production of a copper/zinc containing superoxide dismutase at various stages of development including the adult stage [107].

Cytokines and chemokines found elevated in human serum or pleural effusions of humans infected with *Paragonimus* spp. included thymus and activated-regulated chemokine (TARC), eotaxin, RANTES, and IL-8 [108]. The immune response in general is that of a Th2-dominated response with an IgG4 subclass.

2.4.2.2 Schistosomiasis

This is one of the main human helminthiasis around the world with approximately 200 million people infected worldwide. The main human pathogens are *S. mansoni*, *S. japonicum*, and *S. haematobium*. Two other species, *S. intercalatum* and *S. mekongi*, have been described also in humans although their geographic distribution is more limited [109]. The life cycle [110] starts with penetration of the host's skin by forked-tail cercaria after which they shed their tail and become schistosomulae. At the site of penetration, they induce an inflammatory response known as "swimmers' itch." The schistosomulae then migrate to the portal circulation in liver where they become adults who start to mate for the life of the parasite (3–30 years). Mating adults of *S. mansoni* and *S. japonicum* migrate to the mensenteric venules of bowel and rectum where females start laying eggs that reach the liver and eventually the stools. *S. haematobium* adults migrate to the venous plexus of the bladder where females lay eggs leading to bacterial infections in the bladder, hematuria, and later in the disease process scarring and calcification of the venules. Eggs are shed in the urine. In cases with heavy parasitism, eggs can embolize to other parts of the body including brain and lungs. Once eggs are shed in stools or urine, they hatch in the outside environment and release miracidia which then penetrate the different snails (*Bulinis*, *Biomphalaria*, and *Oncomelania*) in which they develop as two generations of sporocysts. When mature, the snails release the forked-tail cercaria to restart the cycle.

The disease itself is divided into the acute and chronic phases. During the acute phase, the patient develops dermatitis, and the circulation of schistosomulae through the hepatic and pulmonary vascular beds followed by maturation and initial oviposition induces a systemic response that includes fever, chills, sweats, cough, and headaches (only seen in *S. japonicum* or *S. mansoni* exposure) [111]. Lymphadenopathy and hepatosplenomegaly can also be seen. The chronic phase is characterized by a granulomatous response to eggs deposited in the intestinal, portal, or urinary tract veins. Pulmonary schistosomiasis is the result of egg deposition in the pulmonary vascular bed resulting in granuloma formation and fibrosis [111]. If a large area of the pulmonary circulation is involved, secondary pulmonary hypertension can ensue. Eggs reach the pulmonary circulation via urinary veins draining into the inferior vena cava or bypassing the liver through porto-systemic collaterals in cases on *S. mansoni* and *S. japonicum* infection in which liver damage has led to portal hypertension [112].

One of the main driving forces in mouse models of schistosomiasis mansoni behind the formation of liver granulomas once eggs are deposited in the hepatic microcirculation is IL-13 and the IL-13R complex [11]. Liver fibrogenesis is greatly decreased in animals deficient in IL-13 or treated with IL-13 antagonists. It has been shown that IL-13 promotes expression of arginase in myofibroblasts, a step necessary to increase collagen production. Other important mediators include IL-4/IL-4Rα(alpha) and Stat-6, IL-5, and IL-17 [10, 113]. In fact, granulomas evolve in two phases: during the early stages, a short-lived type 1 cytokine response predominates, whereas in later stages a type 2 response takes over and is long-lived [12]. The immune response to the eggs is necessary to neutralize an uncontrolled inflammatory response to egg antigens leading to even death in the early stages of the disease. The most important cell at this stage is the CD4+ Th2 cell. From the parasite's point of view, several molecules have been described as important in skewing the immune response towards the Th2 phenotype. An area of intense research is now focused on the role of several schistosomal glycans including Le^x and LNFPIII conjugates. These molecules act via toll-like receptor-4 or TLR-4 and possibly C-type lectins [114].

In regard to the acute phase of the disease known as "snail fever" or "Katayama fever" or acute toxemic schistosomiasis, it is widely accepted that the signs and symptoms are due to large amounts of circulating immune complexes and elevated levels of proinflammatory cytokines and low type 2 responses [115]. In fatal animal models, an overwhelming type 1 response is usually seen which can be controlled by IL-4 and IL-10. IL-4 in vitro is necessary for development of CD4+ Th2 cells.

The genome sequences of *S. japonicum* and *S. mansoni* have been completed and analyzed. Comparative genomics have also revealed abundant clues of the host-parasite relationship. For example, the schistosome genome has lost approximately 1,000 protein-encoding domains, out of approximately 6,000, most likely due to the parasitic lifestyle. These domains include basic metabolic pathways and defense mechanisms (synthesis of fatty acids, sterols, and purines). At the same time, other gene families have been expanded such as metalloproteases genes which total up to 12 family members in schistosomes and only one orthologue in humans [116, 117]. These metalloproteases are involved in skin penetration and tissue invasion.

Other interesting findings include the presence of genes encoding signaling pathways such as Wnt, Notch, Hedgehog, and TGF-β(beta). Most of the signaling molecules in these pathways (epidermal growth factor, fibroblast growth factor, SAMD, and Ras-Raf-MAPK) have a high degree of homology with mammalian molecules, suggesting that the parasites might utilize the mammalian molecules in addition to theirs [116, 117]. Genes related to immune regulation have also been identified such as cytokine homologs, glycoconjugates, and small lipid moieties, all of which have the potential to subvert the immune system when needed [2].

2.4.3 Cestodes

2.4.3.1 Echinococcosis

This disease is caused by cestodes belonging to the genus *Echinococcus* to which more than five species have been described, namely, *E. granulosus*, *E. multilocularis*, *E. oligarthrus*, *E. vogeli*, and *E. shiquicus* [118]. The first four are well known as human pathogens, but the newly described *E. shiquicus* is of unknown human pathogenicity. *E. granulosus* contains several strains (G1–G10) based on their definitive host isolation [118]. The classic form of the disease (cystic echinococcosis) is due to *E. granulosus*. Another form of the disease known as alveolar echinococcosis is a highly lethal and infiltrative disease in humans caused by *E. multilocularis*. Infections by *E. vogeli* and *E. oligarthrus* lead to polycystic echinococcosis. The latter three are far less frequent in humans due to their host specificity limited to wild animals as opposed to *E. granulosus*. The life cycle of all of them involves two mammalian hosts. Definitive hosts are carnivores in which adult worms live in their intestines. The eggs from adult worms are shed in the environment and when ingested by intermediate hosts (like humans), hatch and liberate embryos that migrate to extraintestinal tissues (liver, lungs, brain, etc.) and turn into metacestode or larval forms. It is these larval forms that are known as hydatid cysts that take different morphologies when causing disease (polycystic, alveolar, or cystic) [119]. In nature, the passage from intermediate host (except for humans) to definitive host is the result of predator–prey interactions between those two hosts.

E. granulosus, as mentioned above, is the most common in humans because dogs are one of the definitive hosts. Infected dogs can harbor up to 40,000 tapeworms which in turn can release up to 1,000 eggs each per day. Fecal-oral transmission is therefore relatively easy if close contact with the dog is present. Indirect transmission via arthropods, fomites, soil, water, and vegetables is also possible. Other hosts besides dogs include sheep, cattle, camels, pigs, and cervids.

Signs and symptoms are extremely variable and depend on the localization of the cyst (lungs, brain, liver, etc.), size of the cyst, and its condition (intact versus ruptured). Conversely, in the brain and eyes, signs and symptoms appear more quickly. Cyst rupture can cause of variety of complications including mild to severe allergic reactions, chest pain, coughing, dyspnea, and hemoptysis. The immune response is usually

biphasic. The first phase is directed against the oncosphere or egg hatching in the intestine and penetrating the gut wall. This response is far more effective in controlling the infection since the metacestode in the extraintestinal tissues has more means of evading the immune response.

Conclusions

The classic triad of infectious agent, host, and environment plays a key role in parasitic as well as in any other infectious agents. The geographic distribution seen with some of the parasitic diseases, especially the ones due to helminthes, is in large part due to the complex life cycles that most of the helminthic parasites need to survive in nature. The interplay between host and parasites is also a very complex system in which virulence factors, developmental cycles, parasite nutrition, host immune response, and immune modulation by the parasite interact in a complicated network that we are beginning to elucidate at the molecular level. The availability of small animal models has provided great insights into the pathogenesis of these diseases. Likewise, with the help of powerful molecular techniques, both in vivo and in vitro experiments can now be designed to elucidate the host-parasite relationship and move the research to a new level.

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General Mechanisms of Tissue Injury in Parasitic Infections

3

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3.1 Introduction

Parasitic infections represent a global health problem, particularly in the developing world [1]. The immunological system is responsible for protecting the host against infection and tissue damage. This protection is normally carried out by holding in proper balance different immune response mechanisms. Chronic infections are associated with cell and tissue injury, and this is influenced by several important populations of cells such as $CD4^+$ and $CD8^+$ T cells, macrophages, dendritic cells, NK cells, mast cells, and eosinophils [2–11]. In 1986 two subpopulations of cells with the CD4 phenotype (T helper (h) 1 and Th2) were identified that control resistance or susceptibility to intracellular parasites [3, 5, 8, 9]. Th1 cells synthesize interferon-γ (IFN-γ(gamma)), interleukin (IL)-2, and tumor necrosis factor (TNF)-α(alpha), and induce antibody production (immunoglobulin (Ig)G_{2a} subclass) in the mouse, macrophage activation, antibody-dependent cell cytotoxicity, and delayed-type hypersensitivity response (DTH), and these cells are also associated with resistance to experimental cutaneous leishmaniasis or exacerbation of helminth infections [8–12]. Moreover, Th2 cells produce a differential set of cytokines such as IL-4, IL-5, IL-6, IL-10 (in the mouse), and IL-13; they provide B cell help for IgG4 (in the human) or IgG1 (in the mouse) isotype switching; they also induce activation of mast cells and eosinophils and are associated with exacerbation of experimental leishmaniasis or resistance to helminth infections [11–16]. Recent studies have shown a larger diversification of this $CD4^+$ T cell effector immunological pathway [17–20]. This knowledge has obliged a reevaluation of the Th1 lineage in immunity to parasites. Those studies that associate the cytokines IL-23 and IL-17 to immune pathogenesis, previously attributed to the Th1 lineage, lead to the discovery of a new population of effector $CD4^+$ T cells currently known as Th17 [21].

Interferon-γ(gamma) is a potent activator of cell-mediated immunity and an inhibitor of cells of the Th2 lineage. Th2 cells evolved to favor elimination of parasitic infections such as helminthes, and this is assisted by production of IL-4,

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IL-5, and IL-13, which are strong activators of B lymphocytes; they induce the synthesis of IgE and induce migration of eosinophils to the gastrointestinal mucosa and trigger mucosal expulsion mechanisms during helminth infections [11, 22–24]. Immune pathogenesis that results from unbalanced Th1 responses to self or commensal floral antigens can promote tissue destruction and chronic inflammation, whereas unbalanced Th2 responses can provoke allergy and asthma [25–29]. A more recently described group of cells known as T reg cells represents cells that produce transforming growth factor (TGF)-β(beta), suppress ongoing T cell responses, and are also associated with inhibition of development of *Leishmania major* (*L. major*) infections in immunologically deficient SCID mice [30–33]. These T reg cells represent a small subpopulation of $CD4^+$ T lymphocytes ($CD4^+CD25^+$ Foxp3 T cells), which develop in the thymus and are dispersed in peripheral lymphoid organs [34]. T reg cells suppress growth as well as the cytotoxic effect of T cells, the secretion of Th1 and Th2 cytokines by effector $CD4^+$ lymphocytes, thus limiting the strength of the immune response, which makes difficult for the host to adequately control infections [35]. Proliferation of T and B lymphocytes that specifically recognize certain parasitic antigens is accompanied by the activation of T reg cells which leads to downregulation of the immune response; repeated infections are not only able to strengthen T cell-mediated immunity by generating memory T cells but can also induce suppressive activity of endogenous T reg cells. Moreover, T reg cells are capable of the direct recognition of microbial molecules, since these cells express Toll-like receptors (TLR)-2, -4, -5, -7, and -8 [36]. Normally T reg cells are anergic, but they are capable of direct proliferation in response to stimulation by TLR ligands also known as pathogen-associated molecular patterns (PAMPS) [37]. T reg cells can also induce a protective immune response to certain pathogens including parasites which leads to their elimination from the host organism [38]. However, an excess of suppression induced by parasites or other pathogens can lead to tissue damage. Therefore, a correct balance between immune reactivity and suppression must be developed in order to eliminate pathogens minimizing self-reactivity.

3.2 Parasitic Infections

It has been recorded by the World Health Organization (WHO) that infection and parasitic diseases contribute approximately one quarter of global disease burden [1]. Parasites induce a number of immunological processes, and the immune response that predominates depends upon the type of the parasite responsible for the infection [8]. In order to illustrate the general principles of immunity to diseases caused by parasites, in this section, we will consider some relevant parasites (targeted by WHO) of man and the main defense mechanisms that control their spread within the host. Parasitic protozoa which infect man include intestinal parasites, protozoa that live free in blood such as African trypanosomes or those that live in erythrocytes such as *Plasmodium* spp., and those that live in macrophages such as *Leishmania* spp., *Toxoplasma gondii* (*T. gondii*), and *Trypanosoma cruzi* (*T. cruzi*) [39, 40]. Parasitic worms that infect man include trematodes, cestodes, nematodes, and hookworms. Examples of these parasites include filarial worms, tapeworms, *Trichinella spiralis* (*T. Spiralis*), *Ascaris* spp., and *Schistosoma* spp. [41].

3.3 Immunity to Parasites and Apoptosis

Humoral and cellular immunity are the two sides of immunity turn on during parasitic infections and ideally are aimed to fight infection. However, in some cases immunity to parasites can exacerbate the disease and induce tissue injury.

3.3.1 Cellular Immunity

Animal models have been most useful to better understand mechanisms of immunity to parasites.

Athymic mice that lack T cells are unable to control several parasite infections. This clearly indicates that T cells play an important role during the development of these infections [39, 40]. In the introduction of this chapter, we described some general aspects of the immune response, including cellular immunity; here we will try to expand this information. There are two major types of lymphocytes that originate in the bone marrow: B lymphocytes mature in the bone marrow, and after activation, they differentiate into plasma cells that secrete antibodies. The second type is known as T lymphocytes which are derived from stem cells in the bone marrow but mature in the thymus; they are further subdivided into two broad types: cytotoxic T lymphocytes ($CD8^+$ T cells) which kill cells infected with viruses or cells that have been transformed into cancer cells, whereas the second class of T cells ($CD4^+$ T cells) are essential in determining B cell antibody class switching and in the activation of macrophages and cytotoxic T cells [22]. Macrophages are cells originated in the bone marrow, and they are one of the three types of phagocytes besides of dendritic cells and neutrophils. Macrophages are distributed throughout the body in different tissues and play an important role in innate immunity. Monocytes differentiate into macrophages upon migration into different organs. Dendritic cells are cells specialized to bind antigen, internalized it, process it, and present it for recognition by T cells [21]. Eosinophils and neutrophils are other important populations of cells involved in parasite destruction. These are cells derived from bone marrow, they are considered important in defense against parasitic worm infections [41, 42]. Pulmonary eosinophilia happens as an undesirable complication in many metazoan infections [41]. Individuals living in Western countries suffer from parasite infections mainly caused by species of *Ancylostoma*, *Strongyloides*, *Toxocara*, and *Ascaris* and filarial infections. Vast majority of nematodes multiply within the human host and provoke pulmonary eosinophilia during larval migration through the lungs. However, there is no permanent lung damage in most cases, although the result is an increased number of eosinophils in the airways or lung parenchyma presenting or not peripheral eosinophilia. Löffler's syndrome is a disease where eosinophils accumulate in the lung as a result of a parasitic infection, although for some authors no matter the cause of pulmonary eosinophilia, they define it as Löffler's syndrome. Visceral larva migrans, a medical condition in children provoked by the migratory larvae of nematodes and tropical pulmonary eosinophilia, are the most common infections that cause pulmonary eosinophilia [42]. The most compromising parasitic eosinophilic lung disease is known as tropical pulmonary eosinophilia. This is a disease caused by the filarial worms *Wuchereria bancrofti* (*W. bancrofti*) and *Brugia malayi* (*B. malayi*); it has been reported that this condition is frequently confused as acute or refractory bronchial asthma [42]. Interleukin-10 and TGF-β(beta)1 are cytokines that induce responses associated with Th17 cells, which are probably effective in the protection against extracellular bacteria and perhaps parasitic infections, but they also play a role in the amplification of autoimmune disorders [18–20] and produce tissue damage by inducing the synthesis of autoantibodies. Future research could be devoted to better understand the role of Th17 cells during nematode, protozoan, fungal, and viral infections and reveal the molecular mechanism of induction of tissue damage by these cells. Neutrophils also known as polymorphonuclear leukocytes (PMNs), they are the most abundant type of leukocytes in the blood stream. They phagocyte pathogens and constitute an important first line of defense against pathogenic bacteria, fungi, and protozoan parasites [43]. Neutrophil cytotoxicity has been demonstrated using PMNs from healthy blood donors that were lethal to uncoated schistosomula in culture media containing complement and antibodies directed against schistosomal antigens. This lethal effect was significantly increased when the enzyme peroxidase from eosinophils (EPO) was bound to the surface of the organisms or when hydrogen peroxide (H_2O_2) and halide ion (Cl^-) were added to EPO-coated parasites [41]. Clearly, during all these cellular and molecular responses, pathogens are also capable of inducing tissue injury by increasing oxidant generation or damaging the oxidative repair systems.

3.3.2 Antibody Responses

During parasitic infections, synthesis of pathogen-specific antibodies is helped by Th cells. These cells assist B cells of each different class to induce antibody production [4, 5, 8, 12, 13]. In addition to the production of specific antibodies, many parasites induce a nonspecific hypergammaglobulinemia due to polyclonal activation of B cells. While Th cells contribute to an increase in specific antibody production, it is expected that much of the increase is due to parasite antigens acting as mitogens or polyclonal inducers for B cells. Protective antibody responses have been demonstrated to act against some parasites [44]. However, polyclonal activation of B cells could induce self-reacting antibodies and tissue injury.

Antibodies are important in the control of extracellular parasites [44]. Thus, antibodies are effective in binding extracellular parasites and preventing the reinvasion of cells but are ineffective once the parasite has been internalized by the host cell. In addition, it has to be taken into account that some parasites such as some African trypanosomes change its antigenic structure in a process known as antigenic variation; this is performed as an attempt to evade the deadly effects of antibodies. There are several molecular and cellular mechanisms associated with antibodies such as complement-mediated lysis and cell-mediated cytotoxicity that operate against parasites [44–46]. Tissue damage might occur when antibodies are raised against parasite antigens and cross-react with molecules present in host's tissue.

3.3.3 Toll-Like Receptors

During the innate immune response, a number of receptors in mammal cells recognize pathogens and trigger cellular innate immune responses [36]. Proteins that recognize molecular features common to many pathogens occur as receptors on macrophages, neutrophils, and dendritic cells and as secreted molecules as well. The binding of specific pathogen ligands by receptors starts rapid responses, which are put into effect without the delay associated with clonal expansion of lymphocytes needed to generate the adaptive immune response. Receptors produced by cells of the innate immune response mediate different functions such as phagocytosis, chemotaxis, production of proinflammatory responses, and intracellular signaling. These functions will contribute to innate immunity and will allow the initiation of adaptive immune responses [36, 37]. Direct binding of pathogens, including intracellular parasites, is carried out through cellular receptors known as toll-like receptors (TLRs). Binding of pathogen-associated molecular patterns (PAMP) by TLRs results in the production and release of cytokines and the production of co-stimulatory molecules needed to activate adaptive immunological responses [37, 47]. There are ten different TLRs in mice and humans that recognize unique PAMPs in pathogens such as parasites.

3.3.4 Apoptosis

Apoptosis is also known as programmed cell death (PCD) and plays an important role in the development of normal tissue as well as in the induction of pathology during different parasitic infections. PCD is responsible for the noninflammatory elimination of harmful or unnecessary cells during embryogenesis and for the correct function and cell renewal in healthy organisms. Differentiation of cells of the immune system and the development of a specific immune response are examples of situations where PCD plays important roles. PCD is a process found in unicellular organisms including some parasites and in cells of more complex organisms. The proper functioning of PCD can occur in living cells presenting features of an organized network which operates through interactions within themselves and/or with their environment. Pathogens, from viruses to parasites, can either delay or induce apoptosis of different types of host cells. PCD is of special interest for defining an adequate immunological response of the host and the mechanisms of pathology following lymphocyte

polyclonal activation during parasitic infections [48]. Elimination of apoptotic cells by phagocytes is an important process during parasite infections and consists of four distinct steps: (1) infiltration of phagocytes at the site where apoptotic cells are located, (2) identification and recognition of dying cells through several molecules and receptor, (3) internalization of dying cells, and (4) processing of internalized cells within phagocytes [49]. Thus, a defective PCD induced by parasite infections could target normal cells and potentially constitute another important process of tissue injury.

3.3.5 Nitric Oxide

A number of cell populations including neutrophils, monocytes, and macrophages produce nitric oxide (NO). Nitric oxide synthesis requires oxidation of a single guanidino nitrogen atom of L-arginine; this process involves the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) and the reduction of molecular oxygen. The enzyme NO synthase (EC 1.14.13.39) (NOS) constitutes a group of enzymes that catalyze the production of NO from L-arginine. The major NOS isoforms can be identified in two groups as constitutive NOS (cNOS) and inducible NOS (iNOS). Constitutive enzymes include two different types known as nNOS and eNOS. The former is neuronal NOS (NOS1) and the latter is endothelial NOS (NOS3) [50, 51]. NO synthesized by the cNOS is involved in many physiologic activities, including neurotransmission and vascular relaxation, whereas inducible NOS (NOS2) is not normally active [50, 52]. In sepsis, which is a deadly complication of visceral leishmaniasis, certain cells are activated by specific proinflammatory compounds such as endotoxin, TNF-α(alpha), IFN-γ(gamma), or IL-1, and this results in the induction of iNOS activity. Cytokines, bacterial endotoxin, and some parasite products may also increase NO production by enhancing arginine availability through many different pathways including the opening of cell membrane channels, the expression of the cationic amino acid transporter (CAT), or by enhancing the amount of a cofactor (tetrahydrobiopterin) needed for NO enzymatic synthesis [50–52]. It is clear that the production of NO must be regulated. However, this regulation process could be affected during parasite infections, and sustained high concentrations of NO will react with superoxide and produce the toxic product peroxynitrite that will lead to tissue destruction through necrotic or apoptotic cell death.

3.4 Immunity and Host-Tissue Damage Induced by Parasites

Some parasites induce tissue damage by production of toxic enzymes such as proteases [52]. Many immune responses developed during parasite infections induce pathology. Thus, tissue-bound immunoglobulins have been found in the muscles of mice with experimental Chagas' disease and in choroid plexus of mice with experimentally induced malaria [53, 54]. Autoantibodies, probably synthesized as a result of polyclonal activation, have been detected against the deoxyribonucleic acid (DNA) of some cell types. Some of those autoantibodies may cross-react with host's tissues and play a role in the development of cardiomyopathy, enlarged esophagus, and megacolon that occurs in patients with chronic Chagas' disease. Thus, these tissue injuries are the result of autoimmune reactions from the host antibodies or cytotoxic T lymphocytes that cross-react with *T. cruzi* [54]. Immune complexes are frequently found during parasitic infections, and their deposition in the kidneys, as reported in the nephrotic syndrome of quartan malaria, may produce several other pathological conditions [54]. Hepatomegaly and splenomegaly of malaria, trypanosomiasis, and visceral leishmaniasis are all associated with increased numbers and activity of lymphocytes and macrophages in those tissues [8, 53, 54]. Fibrosis and the enlarged liver in schistosomiasis are a pathologic consequence of granuloma formation surrounding the worm eggs, similar to a delayed-type hypersensitivity reaction, and can be responsible

of the symptomatology in patients with pulmonary schistosomiasis [55] (see Chap. 4).

Immunosuppression is also frequently found in many diseases such as malaria, Chagas' disease, and diffuse cutaneous leishmaniasis and may lead to concomitant bacterial or viral infections or Burkitt's lymphoma which is sometimes associated with malaria [8, 53, 56, 57].

3.4.1 Granuloma Formation During Parasitic Infections

Once parasites start an infection, they can effectively resist the lethal effects of macrophages and produce chronic infection that can lead to inflammation. Parasites can induce granulomatous inflammation that serves to insulate the pathogens that resist destruction [58]. These granulomas are regulated by T cells that recognize parasite-released antigens. In the tissues, macrophages accumulate and secrete chemicals that induce fibrosis and stimulate the formation of granulomatous tissue and provoke fibrosis. During infection with *Schistosoma* spp., granuloma formation around the eggs is developed [59] (see Chap. 4). Several eggs are transported to the liver where they become insulated behind a capsule containing several different types of inflammatory cells [59]. In experiments performed in mice, granuloma formation predominantly consists of eosinophils and is the result of a T cell-dependent reaction [59]. In visceral leishmaniasis, parasite spread into the liver, spleen, lymph nodes, and bone marrow frequently occurs. This parasite dissemination produces enlargement of the liver and the spleen in a condition known as hepatosplenomegaly; fever, abdominal pain, and weight loss are some symptoms associated with this condition. The disease is fatal if untreated, because of many complications such as secondary infections, anemia, and malnutrition [60]. In visceral leishmaniasis, fever, pallor, weakness, night sweats, anorexia, and weight loss are common and progress to a medical condition known as cachexia (wasting syndrome), a general wasting condition associated with overproduction of TNF-α (also known as cachexin). Children with visceral leishmaniasis can develop diarrhea and growth retardation and, in some cases, can show an oligosymptomatic infection, which usually resolves spontaneously or can develop kala-azar (kala-azar means black fever in Hindi language). Darkening of the skin is not frequently seen although anemia, leucopenia, thrombocytopenia, internal bleeding, and hypergammaglobulinemia are frequently found in this disease. Eventually, untreated disease at any age can provoke cachexia and death [61]. Malaria is a devastating disease worldwide; it has been of interest to immunologists for a long time, as acquired immunity can limit the clinical outcome of infection and can reduce parasite replication. However, reactions in cells of the immune system also contribute significantly to pathology and deaths [62]. Immunopathology in severe malaria comes predominantly from vascular damages resulting from a sequence of events produced by activated effector as well as regulatory cells infiltrating the vascular sites of several target organs such as placenta, bone marrow, brain, spleen, and lungs [62, 63]. One important feature that distinguishes these processes from classical inflammation is the absence of extravasation. Scientific evidence suggests that PAMPs or other compounds produced by parasites induce production of cellular infiltrates consisting of macrophages, neutrophils, natural killer (NK) cells, invariant natural killer T cells (iNKT), $CD4^+$, and $CD8^+$ T cells, leading to local vascular and organ injuries [64]. Cerebral malaria (CM) is a serious complication of this disease. Although, it is worth to mention that previous work on experimental cerebral malaria has identified T and other cells as key mediators of pathology. However, the mechanisms of antigen presentation and induction of several immunological cells therein required to start a pathological process are unknown at the present time [63]. Conventional dendritic cells, but not plasmacytoid dendritic cells, are required for the induction of *Plasmodium*-specific $CD4^+$ T cell responses and subsequent development of experimental CM. This has important implications for the development of malaria vaccines and the therapeutic management of this pathological condition

[65]. It has recently been shown that in a mouse model of cerebral malaria, heme molecules (present in hemoglobin) can produce inflammation and permeabilization of the blood–brain barrier, and this will result in death. The enzyme heme oxygenase-1 or its product (carbon monoxide) can reduce the amount of free heme, opening the way for a new therapeutic approach to this deadly complication [66].

It is clear that in other parasitic diseases such as Chagas' disease, there is not a complete understanding of immunopathology that follows infection. During acute infection, some parasite molecules can activate macrophages, and this provokes nitric oxide synthesis and proinflammatory cytokine production, and as a consequence there is a control of parasitemia and reduction in the number of deaths [54, 67, 68]. Immunity mediated by cells in *T. cruzi* infection is modulated by cytokines, and autoimmunity can be induced and provoke tissue damage. Cellular migration towards target tissues is carefully regulated by chemokines and extracellular matrix components which may represent therapeutic targets to develop novel treatments for this disease [54]. In American trypanosomiasis, it was recently demonstrated that glycoinositol phosphate (GPI) present in *T. cruzi* binds to molecular dimers of TLR2/TLR6 or TLR2/TLR1. This molecular binding induces intracellular signaling which results in the production of nitric oxide, co-stimulatory molecules (CD80, CD86, and CD40), and some proinflammatory cytokines such as TNF-α(alpha) and IL-12. Parasite molecules are presented to lymphocytes, and IL-12 induces the production of IFN-γ(gamma) that will induce the differentiation of Th0 to Th1 cells which in turn stimulates B lymphocytes to synthesize antibodies and CD8 cells [69]. In African trypanosomiasis, soluble variant-specific surface glycoprotein (VSG) and membrane-bound VSG from *T. brucei* induce the production of the adapter protein MyD88 and induce toll-like receptor-dependent signaling and activate the innate immunity against *T. brucei* [70]. African trypanosomes can cause chronic infections through a mechanism of antigen variation whereby they control the humoral immune system of their hosts and escape the lethal effects associated with antibodies such as complement-mediated lysis of parasites. However, besides antigenic variation, these extracellular parasites induce other immunoregulatory activities mainly mediated by innate immunity cells such as macrophages. The modulation of these cells through parasite components and host cytokines has been explored, and it has been found that ribonucleic acid (RNA) levels of TNF-α(alpha) in the IFN-γ(gamma)-primed macrophages were about 100-fold higher as compared to those in the non-primed macrophages. A significant tenfold decrease was observed for IL-10; this clearly suggests a shift to proinflammatory responses in macrophages primed with IFN-γ(gamma) [71].

Waterborne parasitic diseases such as schistosomiasis and giardiasis constantly afflict tropical regions of the world. *Schistosoma*'s eggs are laid in human feces and can contaminate water supplies. Skin-penetrating cercariae constitute the infectious stage for humans and mature within the hosts where they form sexual pairs and produce several hundred eggs every day. A lot of eggs are incorporated into the bloodstream, and in the case of the parasites *S. mansoni* and *S. japonicum*, they stay within hepatic sinusoids and induce a fibrotic granulomatous response [45, 59]. Schistosomiasis experimentally induced in mice has shown the existence of a slightly induced Th1 response to parasite antigens. However, a dominant Th2 response to egg-derived antigens dominates and propagates a fibrotic response within the liver [59, 72]. T helper cell polarization studies have stressed the critical control of Th1, Th2, and Th17 lymphocytes necessary to prevent severe liver pathology. Alternatively activated macrophages which are activated with IL-4 and IL-13 develop in the Th2 environment and normally upregulate some transcription factors such as Ym-1, Fizz-1, and Arg-1 [73]. In granulomatous tissue, these factors are downregulated and IL-13 is reduced, and T reg cells have shown to inhibit experimental formation of Th1 responses to *Schistosoma* parasites [73–75]. In addition, exogenously administered IL-10 inhibits granuloma formation in rodents. Interestingly, IL-10 reduction inhibits

bladder pathology in *S. haematobium*-infected children and adolescents through a TNF-α(alpha)-dependent mechanism induced with egg antigens [76]. Within the liver, T reg cells expressing the phenotype $CD4^+CD25^+$ Foxp3 are recruited [75]; they act as regulators of the immune response and limit immunopathology [45, 59, 74, 75]. The Sm-p40 molecule is known to be a good T cell immunogen. This molecule elicits a clearly immunodominant Th1-biased response which is associated with severe egg-induced immunopathology [74, 75]. Two recently described T cell-sensitizing egg antigens are phosphoenolpyruvate carboxykinase (Sm-PEPCK) and thioredoxin peroxidase-1 (Sm-TPx-1) from *S. mansoni* [76]. These two molecules, but not Sm-p40, induce a balanced Th1/Th2 response in C57BL/6 mice, which develop smaller egg granulomas. These findings in the mouse model suggest that egg antigens can vary significantly in immunogenicity according to the host's genotype. More research is needed to better understand the principal immunogenic egg components to ascertain whether such immunological responses can be manipulated to reduce pathology and have a possible therapeutic application [77].

Cytokines produced by Th2 cells are known to play a significant role in allergy, and they are considered therapeutic targets: they protect against ectoparasites (e.g., that live outside of the human body) and worms that live in the gut, and they suppress inflammation induced by Th1 cells. Th2 cytokines induce mastocytosis, eosinophilia, IgE synthesis, and mucus production, and this type of response protects against some worms [72, 75]. The reciprocal cross-inhibition between Th1 and Th2 cytokines such as IFN-γ(gamma) and IL-4 suggests that it is more effective for the immune system to limit immunopathology by suppressing the inflammatory response not required for host protection against a particular pathogen type than to develop an all-purpose inflammatory response [38]. This, in turn, implies that innate immunity is able to distinguish different classes of parasites but is less efficient to distinguish individual parasites within a particular type. Although, these considerations suggest that a therapeutic approach based on the use of Th2 cytokine antagonists in human therapy may increase the severity of worm infections and increase the risk for Th1 cytokine-mediated inflammatory disorders; however, such treatments should be relatively safer if their use is limited to areas in which worm infections are not frequently presented [38].

3.4.2 Waterborne Parasites and Gut Pathology

Giardiasis is a protozoan infection which is transmitted by contaminated water, and gut-associated lymphoid tissue is needed to clear *Giardia* infections from the gut. T lymphocytes have been shown to be key elements to protect infected hosts against this infection, and this has been shown in experiments where athymic mice that lack T cell responses are infected [78]. In contrast to immunocompetent mice, athymic mice when infected with *G. muris* do not efficiently cure and they develop chronic disease [79], whereas normal mice become resistant to reinfection, and development of chronic disease has been associated with decreased tissue levels of IL-6 [80]. Athymic mice, in contrast, fail to develop immunity to infection with this parasite and are susceptible to secondary infections. Reconstitution of those athymic mice with normal T cells leads to a decrease in parasite number as well as a reduction in additional villus atrophy [79]. Atrophy can also be provoked by activated T cells in the absence of a parasite infection, and these experiments demonstrate that T cells activated with parasite antigens provoke tissue injury [81]. It has recently been demonstrated and reported in the literature that in giardiasis, the loss of intestinal brush border surface area and a considerable reduced activity of the enzyme disaccharidase are mediated by $CD8^+$ T cells. Both types of lymphocytes ($CD8^+$ and $CD4^+$ mesenteric lymph node (MLN) T cells) regulate the recruitment of intraepithelial lymphocytes (IEL), and $CD4^+$ T lymphocytes are responsible for parasite destruction [82]. Additional information from experimental giardiasis in the mouse model suggests a protective role for $CD4^+$ T

lymphocytes, while $CD8^+$ cells are more associated with pathology. In line with these observations, it has been shown that depletion of $CD4^+$ T lymphocytes in mice infected with *G. muris* results in chronic infection [83]. In contrast, infected mice depleted of $CD8^+$ T cells show normal parasite destruction [83]. Considerable infiltration of this lymphocyte subset has also been reported in the small intestine of infected individuals, and it has been suggested that enhancement of cytolytic activity of $CD8^+$ T cells in the intraepithelial compartment of individuals with Crohn's disease may be involved in the induction of epithelial tissue damage [56, 57, 84]. It has been speculated that $CD8^+$ memory cells accumulate in the jejunal mucosa and that cells with the phenotype $CD8^+$ TCR-α(alpha)β(beta)$^+$ are directly involved in the destruction of infected epithelial cells through a perforin/granzyme exocytosis mechanism [85]. They also observed that a Fas/FasL-mediated cytotoxicity may be an indication of ongoing downregulation of immune responses at the local site of the infection by apoptosis [85]. Existing reports have shown that a secondary challenge with fractions of *G. duodenalis* trophozoites of uninfected gerbils may cause deficiencies in the activity of the enzyme disaccharidase [86]. Experimental findings implicating $CD8^+$ T cells, integrins such as alpha(E) beta(7), and possibly TGF-β(beta) in the brush border injury during giardiasis and other pathologic disorders of the gut deserve a further investigation. Whether severity in brush border pathology during giardiasis may contribute to clinical symptoms known in this disease deserves a future investigation. As for the mechanism of microvilli shortening, it has been reported that the alterations of brush border surface section may be associated with reorganization of cytoskeletal actin fibers. Findings from studies performed in vitro have demonstrated that *G. duodenalis* rearranges F-actin and a-actinin in intestinal epithelia [87]. Additional research is needed to clarify the role of T cells on those rearranges during in vivo infections with *Giardia* spp. and to determine whether these effects are directly associated with the shortening of brush border microvilli. It is clear that T cell-mediated immunity plays an important role in the development of gut pathology during experimental and human giardiasis. Once again, the uncontrolled response of this T cell immunity could be detrimental to the host by inducing collateral tissue damage.

Conclusion

Parasitic diseases constitute a major health problem worldwide, affecting mostly poor countries. The World Health Organization has targeted several parasitic diseases as the most important worldwide. These diseases include malaria, Chagas' disease, leishmaniasis, filariasis, and schistosomiasis. Tissue damage and death induced by parasitic infections is produced specially after chronic infections, and several organs are affected depending on the type of infection. Better understanding of the molecular mechanisms of these diseases will be useful to identify targets to develop therapeutic prototypes and vaccines.

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Protozoal Infections

4

Rafael Martínez-Girón

4.1 Introduction

Protozoa (from the Greek *protos*, primitive or original, and *zoion*, animal) are unicellular eukaryotic microorganisms.

Discovery of protozoa can be credited to Anthony van Leeuwenhoek (1632–1723) and Alfred Françoise Donné (1801–1878). Numerous investigators consider Leeuwenhock the father of protozoology, because he discovered with rudimentary microscopes, fabricated by himself, free-living protozoa such as *rotifers* and *vorticella.* On the other hand, Donné is considered the discoverer of *Trichomonas vaginalis*, and being based on Louis Daguerre's works on photography in 1839, he was the first to obtain photomicrographs of these organisms [2].

Protozoa 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, with life cycles of varied complexity, both free-living and parasitic, and, in many cases, a vegetative form (trophozoite) and another resistant form (cyst).

Protozoa involved in bronchopulmonary pathology (Table 4.1) belong to the five phyla established in the taxonomical classification: *Sarcomastigophora*, *Apicomplexa*, *Microspora*, *Myxozoa* and *Ciliophora*.

Bronchopulmonary infestations by protozoa constitute a group of rare diseases [3], but they appear, in most cases, in patients with an underlying clinical condition such as states of suppressed immunity (AIDS, transplants, haematologic malignancies, corticosteroid therapy, etc.). Other high-risk groups for amoebiasis include travellers and recent immigrants [4–6].

Although many of the protozoa that are of medical interest may be grown in culture, this is seldom used for diagnostic purposes, and microscopic visualisation is a simple and cost-effective method, but it implies that the pathologist and cytopathologist should be familiar with the morphology of these microorganisms. Morphologic diagnosis can be achieved either by fresh examination of a specimen or by the use of specific stains (Wheatley's trichrome, Giemsa, Heidenhain iron haematoxylin, Lugol, etc.). Although sophisticated tests including electron microscopy, molecular studies and serology may be available, on a day to day basis, a diagnosis based on standard light microscopy in experienced hands is of great usefulness.

4.2 Pathogenic Agents

As described above and in Chaps. 2 and 3, the mechanisms by which protozoa cause bronchopulmonary pathology may be (1) direct damage to the parenchyma (e.g. toxoplasmosis), (2) through a systemic

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Table 4.1 Protozoa involved in bronchopulmonary pathology

Phylum Sarcomastigophora
Subphylum *Sarcodina*
Superclass *Rhizopoda*
Order *Amoebida*
Family *Endamoebidae*
Genus *Entamoeba*
Order *Schyzopyrenida*
Family *Leptomyxidae*
Genus *Acanthamoeba* and *Balamuthia*
Subphylum *Mastigophora*
Order *Trichomonadida*
Family *Trichomonadidae*
Genus *Trichomonas*, *Tritrichomonas* and *Tetratrichomonas*
Order *Hypermastigida*
Family *Hypermastigidae*
Genus *Lophomonas*
Order *Kinetoplastida*
Family *Trypanosomatidae*
Genus *Leishmania* and *Trypanosoma*
Phylum Apicomplexa
Class *Sporozoa*
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*

inflammatory response by haematogenous dissemination (e.g. malaria) and (3) by contiguity with an adjacent lesion (e.g. amoebiasis) [7].

4.2.1 Phylum Sarcomastigophora

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

Sarcodina subphylum is characterised by mobile trophozoites, inconstant in shape, in the form of pseudopods, although some may present temporary flagellae. In the *Mastigophora* subphylum, the trophozoites are constant in shape and have one or multiple flagella, although on occasions with other associated organelles, both at the base and as undulating membranes.

4.2.1.1 Genus *Entamoeba*

Several species belong to this genus, although only six (*E. histolytica*, *E. dispar*, *E. moshkovskii*, *E. polecki*, *E. coli* and *E. hartmanni*) are related to the human intestinal lumen [8] and one more (*E. gingivalis*) to the oral cavity. Nevertheless, the leading species traditionally recognised as a pathogen for humans is *Entamoeba histolytica*. Humans are *Entamoeba histolytica* principal host and reservoir. 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. Visits to endemic areas (Africa, Asia and Central America) and immigration are two additional risk factors to be taken into account.

E. histolytica is the agent responsible for many clinical cases of traveller's diarrhoea and is also the causative agent of amoebic diarrhoea and amoebic dysentery, all of which are consequences of infection of the large intestine. Extraintestinal amoebiases are due to trophozoites going into the blood vessels and then being carried in the blood stream to various organs such as the liver, lung, brain, heart and skin.

Trophozoites (Fig. 4.1) may be recognised by a series of morphological features such as amphophilic and vacuolated cytoplasm, amoeboid in

shape; they are variable in size (10–60 μm) and frequently contain phagocyted erythrocytes. The nucleus possesses a finely dispersed chromatin and a prominent karyosome.

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 due to fistulisation [9–11], with the lower lobe of the right lung being the area most frequently affected. However, haematogenous dissemination has also been described as a cause of pulmonary lesions due to *E. histolytica* [12].

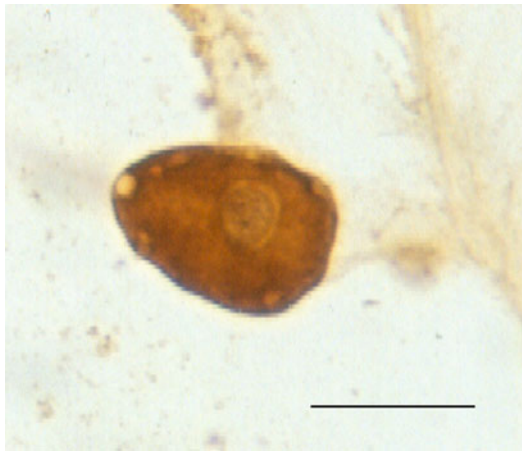

Fig. 4.1 Trophozoites of *Entamoeba histolytica*. *Entamoeba histolytica* trophozoite in an iodine-stained wet mount sample (scale bar 12 μm)

The clinical findings depend on the associated pulmonary pathology, frequent symptoms being fever, productive cough, haemoptysis and pleuritic chest pain [13, 14]. Occasionally, hepatic abscess may cause venous compression, respiratory distress and alterations in arterial gasometry [15]. In addition, amoebic pulmonary abscesses may cause superior vena cava syndrome [16, 17]. 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 is a highly suggestive sign [18].

Amoebic lung abcesses may vary in size, from microns up to several centimetres. As in other locations (Fig. 4.2), they are usually thick walled and formed by a necrotic core surrounded by a viable oedematous tissue with a mixed inflammatory cell infiltrate (neutrophils, lymphocytes and histiocytes) and the presence of trophozoites. Direct examination of the pus can demonstrate the trophozoites [19].

Hepatobronchial fistula, pleural effusion and bronchopleural fistula with pyopneumothorax (Fig. 4.3) are other lesions described.

In addition to the gross and histopathological examination, sputum cytology or fine-needle aspiration has proved their usefulness in the diagnosis of pulmonary lesions in which the diagnosis of pulmonary amoebiasis was not initially suspected [20–23]. Moreover, in cases of pulmonary amoebiasis without data indicating

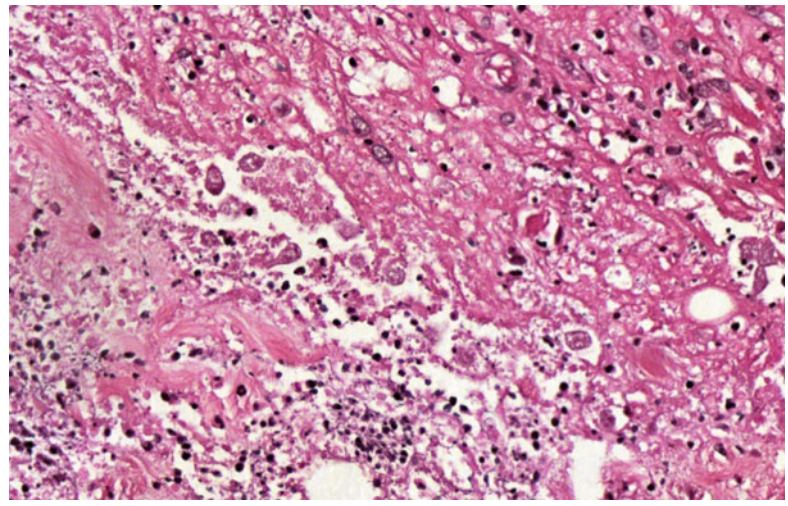

Fig. 4.2 Amoebic colon abscess. Among the necrotic debris, some well-preserved trophozoites are observed (H&E, ×200) (Courtesy of Dr. Mirta Garcia Jardon, South Africa)

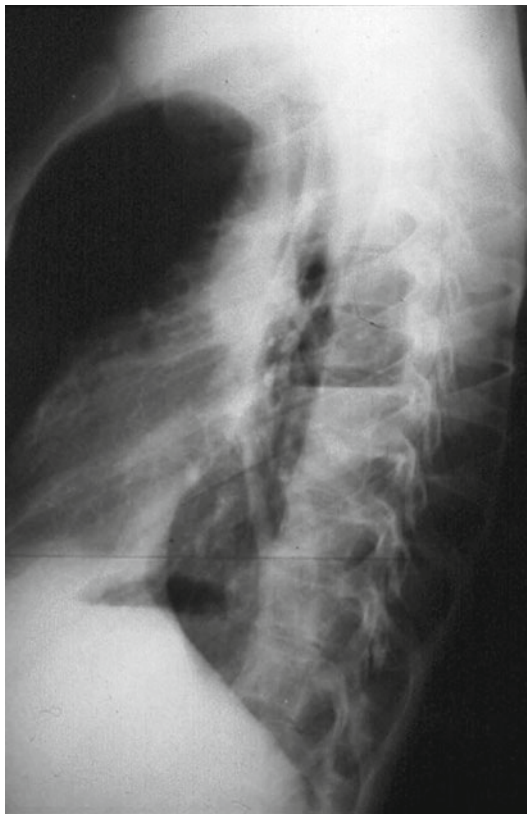

Fig. 4.3 Pulmonary amoebiasis. Lateral chest radiograph showing a left pleural effusion with an air-fluid level due to a bronchopleural fistula

intestinal and/or hepatic repercussions, which have to be distinguished from other processes such as bronchial carcinoma, pulmonary tuberculosis, lung abscess and empyema [24], the combination of trophozoite visualisation through cytological sputum examination with DNA extraction techniques has proved to be of great utility [25].

Another species included in this genus is *Entamoeba gingivalis*. It is considered as a harmless commensal in the oral cavity and found in gingival tissues around the teeth associated with poor oral hygiene [26]; however, this protozoon has been reported as cause of periodontal disease [27] and lung abscess [28]. *E. gingivalis* has been described also in sputum samples, and it is important to note that it may be confused with *E. histolytica* [29, 30].

4.2.1.2 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" [31]. Knowledge of these protozoa is important for two reasons: on the one hand, they cause pathologies in various organs (brain, cornea, skin, lung, etc.); on the other, they may harbour and transmit pathogenic bacteria, some of which, such as *Legionella pneumophila* [32, 33], *mycobacteria* [34, 35], *Chlamydia pneumoniae*, *Parachlamydia acanthamoebae* [36, 37] and *Francisella tularensis* [38], have important repercussions on the respiratory system. This last factor may have clinical relevance, as a series of microorganisms associated with amoebae are mentioned as agents causing nosocomial pneumonia [39].

Of the genera known at present, only four are associated with human pathology: *Acanthamoeba*, *Balamuthia*, *Naegleria* and *Sappinia*.

Studies carried out on experimental animals [40–42] have shown that certain species such as *N. fowleri*, *A. castellani* and *A. polyphaga* may cause direct damage to the pulmonary parenchyma in the form of confluent necrotising pneumonitis, with thickening of the alveolar walls, mononuclear inflammatory infiltrate and development of hyaline membranes in the alveolar spaces. Moreover, with regard to other Acanthamoeba species (*A. lenticulata*), histopathology of brains and lungs revealed marked tissue necrosis and haemorrhagic lesions with massive proliferation of amoebae [43].

In the case of humans, only *Acanthamoeba* spp. and *Balamuthia mandrillaris* have been related with pulmonary pathology [44–48]. Both *Acanthamoeba* and *Balamuthia* trophozoites, in infected tissues, pose a granular and vacuolated cytoplasm, ovoid in shape (15–35 μm in diameter for *Acanthamoeba* and 15–60 μm for *Balamuthia*). Nucleus is round and with a prominent central karyosome.

Pneumonitis and diffuse areas of alveolar consolidation are the most frequent lesions, with the

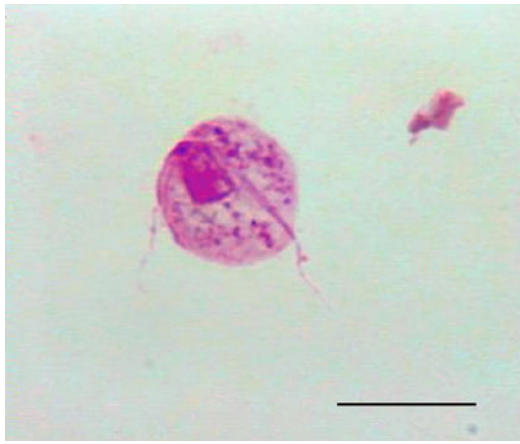

Fig. 4.4 *Trichomonas vaginalis* trophozoite (Giemsa stain, ×1,000) (Courtesy of Prof. Oscar Jodra, Spain)

presence of cystic forms and trophozoites in the alveolar spaces. The identification of trophozoites in bronchoalveolar lavage samples and their subsequent cultivation help establish a diagnosis [49].

In other types of samples, such as nasal exudates, the presence of amoebas (*Naegleria* sp. and *Acanthamoeba* sp.) has been described [50].

Although molecular techniques are not available in most of diagnostic laboratories, they have a high specific and sensitive for the detection of these protozoa in clinical and environmental samples [51].

4.2.1.3 Genera *Trichomonas*, *Tritrichomonas* and *Tetratrichomonas*

Three species of *Trichomonas* that are pathogenic in humans (*T. vaginalis*, *T. tenax* and *T. hominis*) are implicated in bronchopulmonary pathology. Morphologically, these species have similar shape (ovoid or "pear-like"), with four free anterior flagella and other posterior one attached to an undulating membrane (Fig. 4.4).

Pulmonary infection by *T. vaginalis* may appear in newborn babies [52, 53], adults presenting suppressed immunity [54, 55] and in patients with acute respiratory distress syndrome [56, 57]. In the case of newborn babies, there exists the antecedent of vaginal birth by mothers infected with the protozoon [58, 59]. Complications may be immediate, in the form of respiratory difficulty [60], or long term [61]. Detection of the presence of mobile forms of the microorganism in secretions of the respiratory tract or from necrotic tissues 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, the appearance of this microorganism is the most frequent cause of the development of a pulmonary pathology [62]; factors that predispose towards its development are poor dental hygiene, malnutrition, alcoholism and prior debilitating or pulmonary diseases (carcinoma, abscess, bronchiectasis, etc.) [63, 64]. In these cases, pleural effusion and empyema discharge is the most common complication [65–68], 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 [69].

Identification of these species is possible using techniques of molecular biology [70–72].

As for *T. hominis*, also called *Pentatrichomonas hominis*, a microorganism 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 [73, 74].

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.

The possibility has recently been suggested that some of these species, originating in animals, may have become adapted to humans. Proof of this are two works that, by means of biological tests, have demonstrated genetic sequences of these protozoa in samples from the human respiratory apparatus [75, 76], being observed also in a case of empyema [77]. These findings have been recognised as opening up an important new field of research [3].

4.2.1.4 Genus *Lophomonas*

Lophomonas is a genus belonging to the suborder *Lophomonadina* (*Hypermastigida* order). 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 Chinese literature, two works are mentioned in which protozoa of the *Hypermastigida* order are related to pulmonary pathology, responding to metronidazole therapy [78, 79]. The specie *Lophomonas blattarum* has been reported as causative agent implicated in four cases of patients undergoing renal transplant who developed pulmonary pathology [80] and in other case with radiological findings consisting in multiple patchy opacities scattering in both lungs accompanied with bronchial obstruction [81]. A bronchial biopsy showed infiltration of neutrophils and eosinophils in the bronchial wall with necrotic areas. In a transbronchial direct smear, a large number of living protozoa were found. In all these cases, too, therapeutic response with metronidazole was demonstrated.

L. blattarum is a multiflagellated protozoon, round to oval in shape (20–60 μm in diameter), and has a peculiar apical tuft of numerous flagella (Fig. 4.5). In our experience, we have had the opportunity to observe it in the sputum from two asthmatic patients [82, 83].

It is very important to highlight that, however *L. blattarum* can be identified in the respiratory samples, an observer with insufficient experience with the microscope is possible to mistake flagellated protozoa for detached bronchial ciliated cells [84].

Uncommon protozoa not so far taxonomically classified but which, from their morphological and staining characteristics, are tentatively catalogued as belonging to the *Hypermastigida* order (Fig. 4.6) have been described in the sputum of asthmatic patients [85], nasal secretions of patients with allergic rhinitis [86] and sputa of immunosuppressed patients, especially those with AIDS [87, 88].

A striking fact is the finding of similar flagellated protozoa in intestinal extracts of dust mites and cockroaches, establishing a possible nexus between these microorganisms and respiratory allergy [89, 90]. About this topic, we have postulated the implication of these uncommon multiflagellated protozoa, as disrupters of the airway epithelium, in asthma pathogenesis [91, 92].

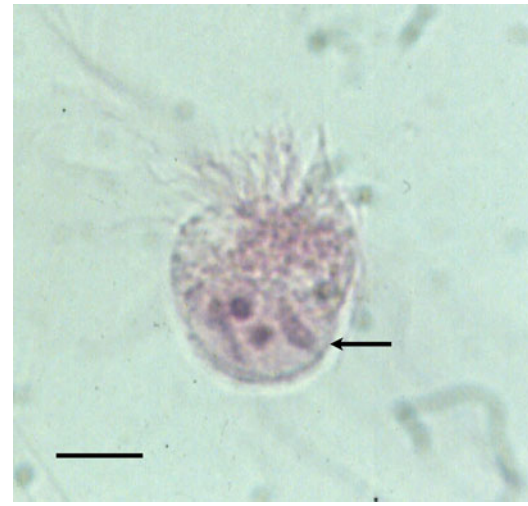

Fig. 4.5 Lophomonas blattarum in a sputum smear. Note the peculiar arrangement of the flagella and the nucleus indicated by the *black arrow* (Papanicolaou stain, ×1,000. Scale bar = 10 μm)

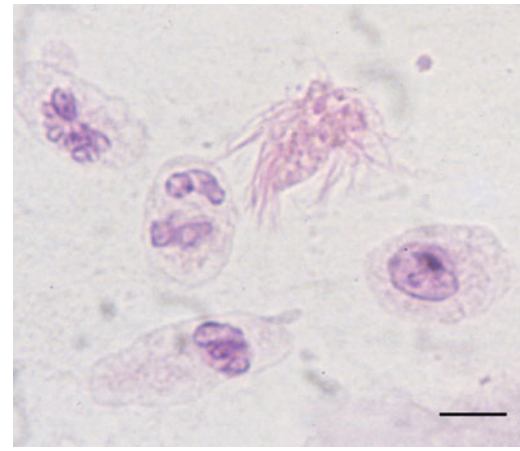

Fig. 4.6 Multiflagellated protozoa (probably belonging to the *Hypermastigida* order) on sputum smear from an asthmatic patient (Papanicolaou stain, ×1,000)

4.2.1.5 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 coast), with a biological cycle

integrated by an extracellular form or promastigote, the infective stage (14–20×2 μm in size, fusiform in shape, with a central nucleus in position and a flagellum) in the intestinal tract of phlebotomes (small insects similar to mosquitoes), and an intracellular form or amastigote (oval in shape, 2–3 μm in size and with a very small nucleus) 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 [93].

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 [94, 95].

In animal experimentation models [96], the pulmonary lesion caused by *Leishmania* sp. is characterised by chronic diffuse interstitial pneumonitis with thickening of the alveolar septa due to the depositing of collagen and a cellular exudates formed principally by macrophages, lymphocytes and plasmatic cells. A similar pulmonary pathology involving foci of septal fibrosis and interstitial pneumonitis with a mononuclear infiltrate has also been described in humans [97, 98]. In these cases, amastigotes of *Leishmania* may be observed in histiocytes of the lung interstitium. Other forms in which the disease presents itself are granulomatous inflammation of the bronchial mucosa and mediastinal lymph nodes [99] and the development of pleural effusions with the presence of the intracellular protozoon in macrophages [100, 101]. In other types of samples of the respiratory apparatus, such as transbronchial biopsy [102] and bronchoalveolar lavage [103, 104], 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 [105, 106], although cases have also been described in immunocompetent patients [107], and in these latter, differential diagnosis with respect to neoplastic processes has been proposed [108]; 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 [109].

Direct observation of the parasite 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, especially in AIDS patients [110].

4.2.1.6 Genus *Trypanosoma*

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 American trypanosomiasis. It is this latter which may affect the respiratory apparatus, either in organs such as the heart and oesophagus [111] or during pregnancy through the placenta (the congenital form of the disease).

Trypanosomas have three stages in its life cycle: trypomastigote, epimastigote and amastigote. Morphologically, they are approximately 20 μm in long and generally slender. They have a thin, irregularly shaped membrane and a centrally positioned nucleus. A flagellum stems from the kinetoplast and runs through the remainder of the parasite and also extends beyond it (Fig. 4.7).

In a wide study of post-mortem cases [112], 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.

In experimental animals, it has been demonstrated that parasitaemia due to *T. cruzi* is the cause of pneumonitis [113], 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 microorganisms was observed in the walls of the bronchia accompanied by an inflammatory reaction. Microorganisms also appeared in the walls of the large vessels. Same findings are also

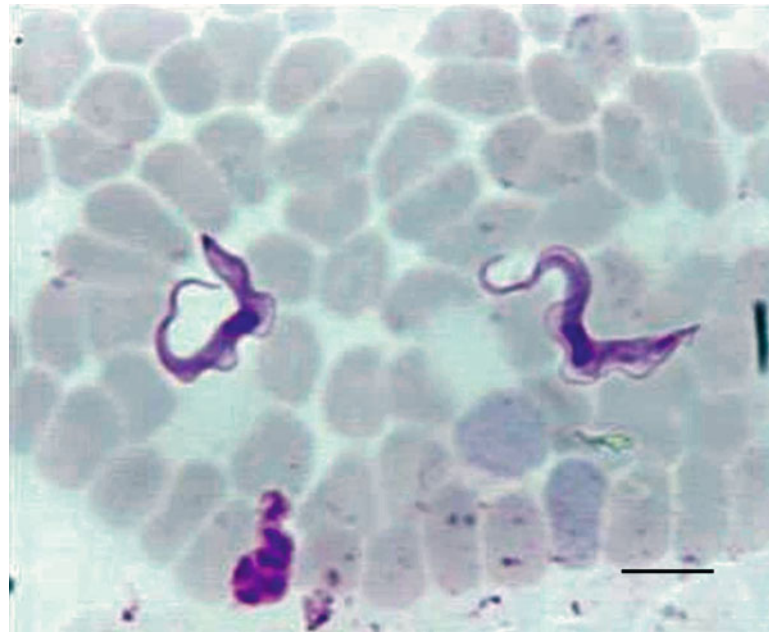

Fig. 4.7 *Trypanosoma brucei gambiense*. Giemsa-stained trypomastigotes in a thin blood film (×1,250) (Courtesy of Prof. Oscar Jodra, Spain)

observed in human pathology, with the presence of thickened septa containing numerous histiocytes and obliterated alveoli. Numerous amastigotes may be observed in histiocytes and the alveolar walls.

In cases studied of the congenital form of the disease [114], pneumonitis is the most frequent lesion, with the presence of amastigotes in lungs, placenta membranes and the umbilical cord.

During the acute phase of Chagas' disease, numerous parasites appear in peripheral blood that can be detected by direct parasitological tests. 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. 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 [115, 116].

4.2.2 Phylum Apicomplexa (Sporozoa)

Protozoa belonging to this phylum 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 [117].

Various genera of this phylum may cause pulmonary pathology:

4.2.2.1 Genus *Cyclospora*

Of the various species belonging to this genus, only *Cyclospora cayetanensis* is pathogenic for man. The microorganism is acquired following the ingestion of sporulated oocysts through contaminated food and water [118].

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 the effects of *Cyclospora* on the respiratory apparatus have not so far been identified, two cases have been documented on the

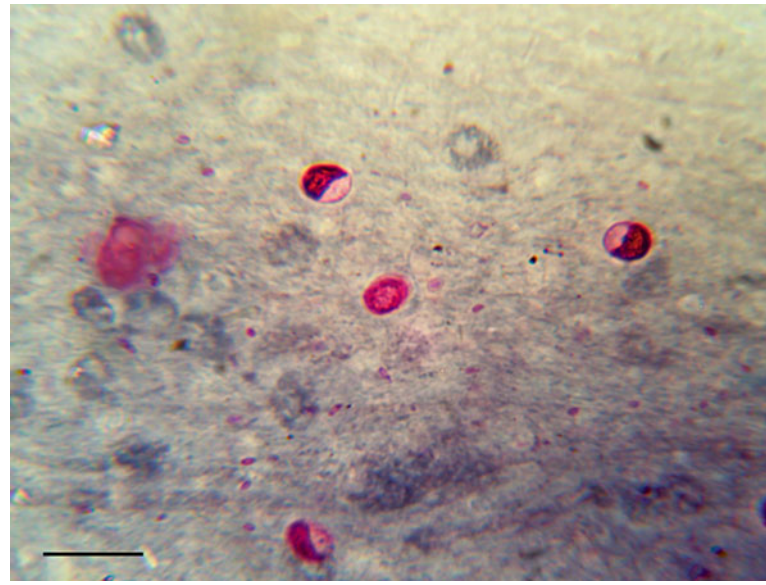

Fig. 4.8 Acid fast stain for *Cryptosporidium parvum*. Oocysts appear in red (×1,000, scale bar = 10 μm) (Courtesy of Prof. Oscar Jodra, Spain)

presence in sputum [119, 120] of oocysts, corresponding to the species C. *cayetanensis*, with concomitant infection, in both cases, by tuberculosis.

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, safranin, calcofluor white, etc.). Because to the limitations of microscopic diagnosis to detect oocysts, methods based on the PCR are highly specific [121].

4.2.2.2 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* [122].

In man, the infection is acquired by the ingestion of oocysts (Fig. 4.8) through water and food contaminated with faecal matter, contact with animals or from person to person [123].

The principal clinical expression of cryptosporidiosis is watery diarrhoea of variable duration, the process being self-limiting in immune competent persons and tending to become chronic in immune depressed patients, among whom it may prove fatal. It is among this latter group that clinical forms of extraintestinal repercussions may be observed [124].

All the cases of invasion of the respiratory apparatus by *Cryptosporidium* that have been documented correspond to immunodepressed patients, the principal cause being the appearance of AIDS [125–133], although other processes also exist, such as those corresponding to malign haematological pathologies [134] and bone marrow transplant recipients [135, 136].

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 are proposed as possible routes [137]. Novel findings suggest the potential for respiratory transmission of cryptosporidiosis, as it has been reported in HIV-seronegative children [138].

Studies in experimental animals subjected to immunosuppression have demonstrated that *Cryptosporidium* may directly damage the respiratory epithelium [139–141], especially outstanding 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 microorganisms 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 microorganisms.

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, although the autopsy confirms the presence of *Cryptosporidium* in lung tissue, it is sometimes difficult to confirm its implication in death due to the fact that other pathogens are also present in the bronchial tree.

The presence both of non-cystic 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 [142–144]. 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 [145].

4.2.2.3 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 [146]. Trophozoites (tachyzoites) of *T. gondii* are arched in shape (4–8 × 2–3 μm) with a nucleus at the blunt posterior end. Cysts of *T. gondii* are usually spherical and range in size from 5 to 50 μm in diameter.

In healthy persons, toxoplasmosis causes asymptomatic infection or subclinical forms (fever and lymphadenopathies). The most serious forms are being 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 as an acute pneumonia [147–149], 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 [150, 151]. Apart from AIDS [152–157], other processes such as malign haematological pathologies [158] and organ transplants are also important causes of pulmonary toxoplasmosis [159–166]. 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 [167, 168], the levels of gamma interferon 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 Th1 lymphocytes in the lung in cases of reactivation of the infection by *T. gondii* [169].

The most frequent clinical symptoms are fever, cough, laboured breathing, tachypnea and dyspnoea, with this latter, in some cases, leading to a hypoxemia with ARDS, which is one of the main causes of death among the patients [170–173].

Anatomopathological examinations carried out, either in pulmonary biopsies or during necropsies, show lesions relating to the state and intensity of the infection [155]. Macroscopically, the lungs appear congested, with petechial haemorrhages and areas of consolidation. Histopathology shows interstitial pneumonitis (Fig. 4.9) with inflammatory lymphocyte infiltrate, diffuse alveolar 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 parenchymatous necrosis and the presence of numerous tachyzoites, both extra- and intracellular. In the cytological smears prepared

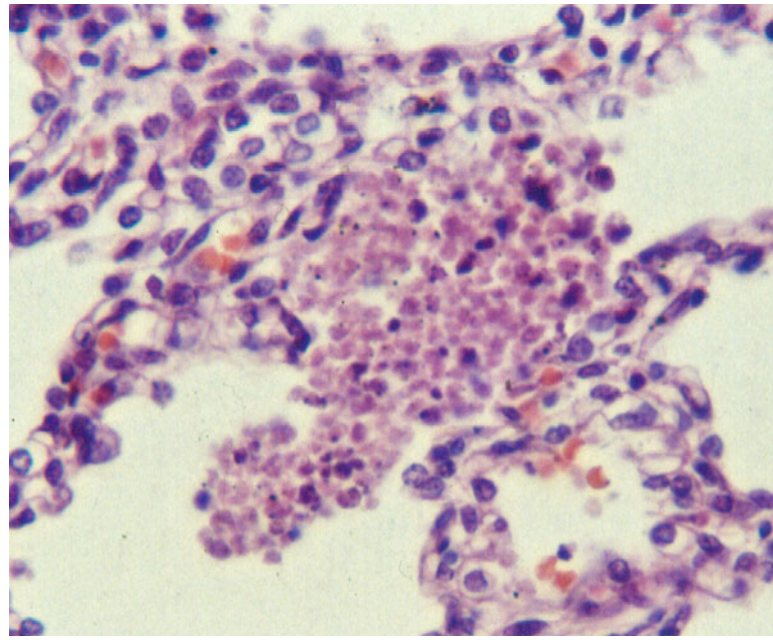

Fig. 4.9 Interstitial pneumonitis in pulmonary toxoplasmosis. Numerous trophozoites in the alveolar spaces are observed (HxE, ×400)

from pleural effusions and stained with the May-Grünwald-Giemsa technique, the visualisation of many tachyzoites located extracellularly and intracellularly of polymorphonuclear leucocytes and macrophages is described [174, 175].

Various diagnostic procedures permit visualisation of the parasite in samples from the respiratory system, one of the most significant being bronchoalveolar lavage [176–179], with Giemsa staining of the samples. In the sputum, there also appears *T. gondii*, this being an alternative if invasive techniques cannot be carried out [180]. In cases in which infection is suspected, or in which the microorganisms are not observed, techniques such as PCR may be of great use [181, 182].

4.2.2.4 Genus *Plasmodium*

This genus comprises the intracellular protozoa that are responsible for malaria. 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, which has an evolutionary cycle consisting of an asexual stage in man, with an extraerythrocytic and an intraerythrocytic phases and a sexual stage that develops in the mosquito.

In countries of our environment, malaria is one of the principal imported acute diseases with pulmonary manifestations [183, 184].

P. falciparum and *P. vivax* are the species that most frequently affect the lungs, while *P. ovale* and *P. malariae* are the rarest [185]. The collectives principally affected are children, pregnant women and travellers to countries where the disease is endemic [186].

Pulmonary oedema is the principal manifestation of the effects of malaria on the lung [187], especially in cases involving *P. falciparum* and to a lesser extent *P. vivax* [188]. The increased permeability of the alveolar capillaries appears to be the principal mechanism by which the plasmatic liquid fills the alveolar spaces [189, 190]. "In vitro" experimental models have demonstrated that the cytoadherence of the infected erythrocytes to the vascular epithelium, through various molecules (ICAM-1, E-selectin, VCAM-1, etc.), is one of the main factors provoking capillary damage with subsequent increase in permeability [191–193]. On the other hand, a longitudinal study in adults has suggested that *P. vivax-infected* erythrocytes may sequester within the pulmonary microvasculature [194].

Another frequent pulmonary complication of malaria is acute respiratory distress syndrome in which are implicated both *P. falciparum* [195–197] and *P. vivax* [198–201].

In the anatomopathological examination, the lungs appear, macroscopically, congested and oedematous, with numerous haemorrhagic focal areas. The coexistence of pleural and/or pericardial discharge may be observed. In the histological sections, pleural oedema, capillary congestion, hyaline membranes and thickening of the alveolar septa are evident. The presence of a brown pigment called "hemozoin" or malarial pigment may be observed in the interior of the alveolar macrophages. As an autopsy finding, parasitised erythrocytes in the blood capillaries may be also observed [202].

A case of obliterating bronchiolitis with organisational pneumonia associated to *P. vivax* is described in the literature, with therapeutic response to corticoids [203].

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 [204].

4.2.2.5 Genus *Babesia*

Babesias are intraerythrocytic protozoa of which there exist various species, being of interest for humans, fundamentally, *B. divergens* and *B. microti*. Their principal reservoirs are cattle and wild rodents. Man acquires the infection (babesiosis), characterised fundamentally by the presence of fever and haemolysis, through tick bites.

Among the factors of risk for systemic infection are immunosuppression, advanced age, antecedents of splenectomy and the transfusion of contaminated blood products from asymptomatic donors [205]. 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 [206]. Among the clinical manifestations are fever, cough and laboured breathing, with non-cardiogenic pulmonary oedema being the most frequent development [207]. Among the causes of this pulmonary complication may be the lack of deformability of the infected erythrocytes as they pass through the pulmonary capillaries and an increase in their adhesiveness to the vessel walls.

Some cases of adult respiratory distress syndrome developed as a complication of the disease have been reported [208, 209]. In one of the cases, the post-mortem findings showed marked congestion and pulmonary consolidation. In the histological sections, hyperplasia 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 intraerythrocytic parasites in peripheral blood. Although Babesia may be confused with Plasmodium, some morphological characteristics such as pleomorphism (very typical gemmated or "Maltese cross" shapes) and vacuolation help to differential diagnosis. On the other hand, molecular techniques allow a more specific diagnosis [210].

4.2.3 Phylum Microspora

Microsporidia are intracellular obligate parasites (they lack organs such as mitochondria and Golgi's apparatus). Because its genomic biology is atypical, new data seems to indicate that they are related to fungi-like organisms [211]. Its 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 in diameter and oval morphology) as the infecting forms [212]. Of the eight kinds implicated in human pathology, the most frequent species are *Enterocytozoon bieneusi*, *Encephalitozoon cuniculi*, *Encephalitozoon hellem* and *Encephalitozoon intestinalis*.

In immunocompetent person, microsporidiosis may be an asymptomatic infection, or cause mild diarrhoeic episodes, although it is in immunodepressed patients where the infection acquires particular importance, the most frequent form being gastrointestinal, although multiorganic repercussions also exist [213, 214]. Oral, ocular, sexual and respiratory as transmission routes are described, being this late confirmed by the observation of microorganisms in the sputum and into the tracheobronchial tree [215, 216].

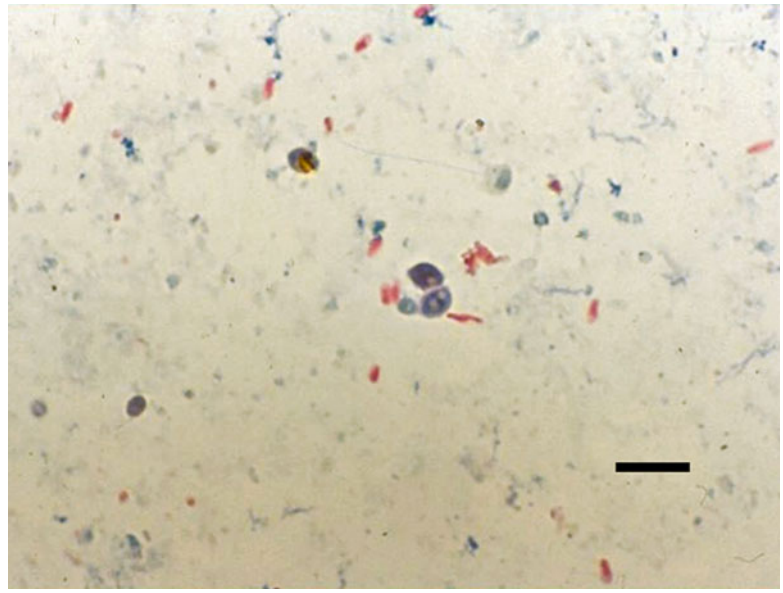

Fig. 4.10 *Encephalitozoon hellem*: spores stained with a modified trichrome stain in a bronchoalveolar lavage (×1,250) (Reprinted with permission from Martinez-Giron et al. [7])

All the cases of pulmonary microsporidiosis documented correspond to immunodepressed patients, the predisposing factors being AIDS [217–219] and transplant recipients [220–224]. Among the possible mechanisms by which the microorganisms colonise the respiratory apparatus are inhalation, regurgitation, orofaecal contamination and haematogenous dissemination from an intestinal focus.

Among the clinical manifestations of infection of the respiratory apparatus by microsporidia are rhinosinusitis, fever, persistent cough, dyspnoea and acute respiratory distress that, in some cases, developed before the patients' death due to cardiorespiratory failure [225, 226].

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.

The samples in which the microorganism could be identified correspond to nasal secretions, sputa, tracheobronchial aspirate and bronchoalveolar lavage (Fig. 4.10), on which it was possible to carry out staining (Weber's modified trichromic and calcofluor), immunofluorescence, electron microscopy and specific cell cultures [227, 228]. Also, the use of molecular techniques, much more sensitive and discriminative, will contribute to determining the true prevalence of microsporidiosis and to better management of infected immunocompromised patients [229].

4.2.4 Phylum Ciliophora (Ciliates)

Protozoa belonging to this phylum have as fundamental feature 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 genus known to produce pathology in humans is *Balantidium coli*. This protozoon, depending on of its life cycle, possesses two morphological stages (cyst and trophozoite). In its trophozoite stage (Fig. 4.11), *B. coli* is one of the largest protozoa (50–300 μm in length), with an ovoid body covered with cilia, a typically large kidney-shaped macronucleus and a less conspicuous micronucleus. In its cyst stage, the parasite takes on a smaller, more spherical shape.

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,

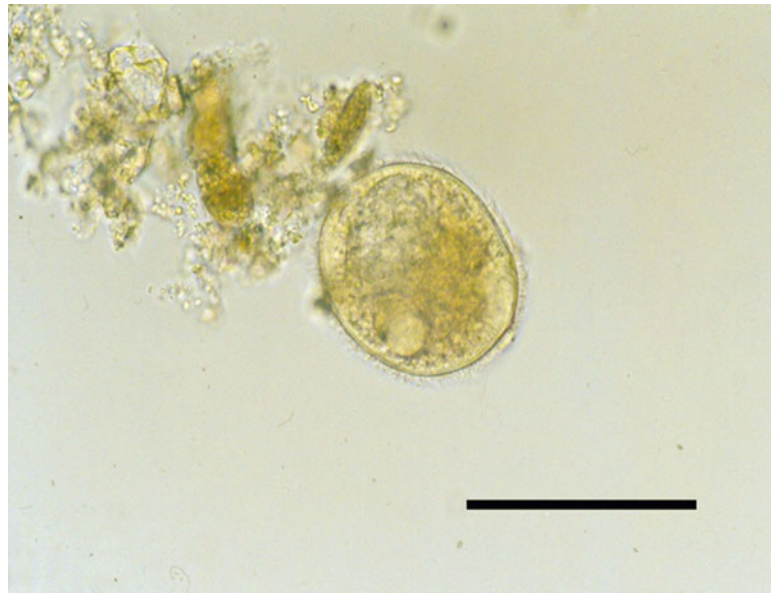

Fig. 4.11 *Balantidium coli* trophozoite in a wet mount stained with iodine (×500) (Reprinted with permission from Martinez-Giron et al. [7])

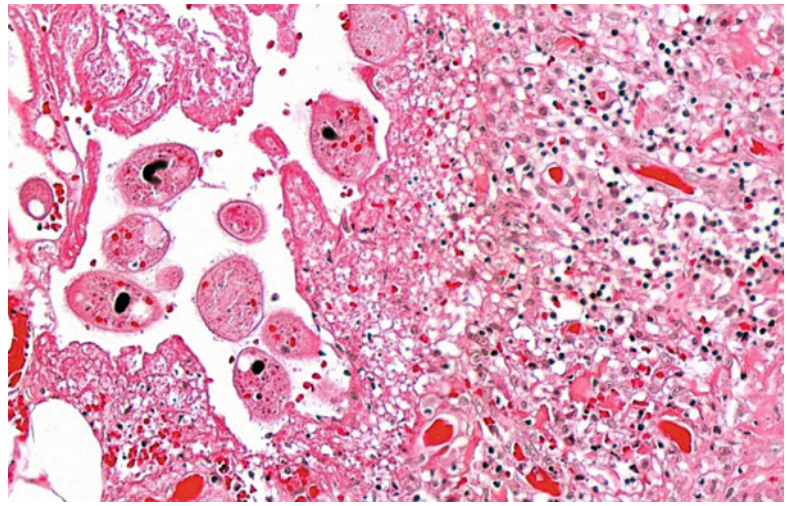

Fig. 4.12 Colonic abscess due to balantidiosis. In the image it is possible to observe some trophozoites with the macronucleus and a vacuolated cytoplasm surrounded by cilia (HxE, ×400) (Courtesy of Dr. Mirta Garcia Jardon, South Africa)

an important factor would seem to be some relation with pigs. Even so, person-to-person transmission should also be considered. Infection in man fundamentally affects the colon, provoking clinical situations of variable intensity, ranging from asymptomatic forms to severe diarrhoea with ulceration of the mucosa [230].

Pulmonary infection by *B. coli* is quite rare, with few cases described in the literature. Among the predisposing factors are, mainly, immunosuppression [231, 232] and contact with pigs [233–235], although a case has also been described of a patient with antecedents of asthma and not immunodepressed [236] and, other, also in an immunocompetent man without history of intestinal disease, with lung involvement due to a severe pulmonary haemorrhage and respiratory failure [237].

Although the mechanism by which the lungs are affected is not completely clear, haematogenous and lymphatic dissemination by ulceration of the intestine (Fig. 4.12) 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, non-productive and persistent cough, dyspnoea of variable intensity (one of the patients required assisted respiration) and thoracic pain.

In the examination of fresh samples, whether obtained by aspiration or by bronchoalveolar lavage, and in bronchial biopsy also, the presence of numerous trophozoites is described.

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Nematoda

5

Roberto Barrios and Abida K. Haque

5.1 Introduction

The diagnosis of helminthic infections can be challenging [1]. Helminths are subdivided into roundworms (nematodes) and flatworms (platyhelminths). Nematodes are characterized by having a cylindrical body and a body cavity known as pseudocoelom or pseudocele [2]. The main groups of nematodes of clinical importance can be classified as Rhabditia, Strongylida, Oxyurida, Ascaridia, Spirurida, and Trichuroidea [3].

Rhabditia: The rhabditia are characterized by free-living existence, but some species have a parasitic phase such as Strongyloides stercoralis which is clinically important.

Strongylida: Male members of this family have a bursa at the posterior end and are known as hookworms; the genera *Ancylostoma*, *Necator*, *Trichostrongylus*, and *Angiostrongylus* have one or more species that is parasitic in humans.

Oxyurida: Oxyurida has only one species, but it is a common parasite in humans: *Enterobius vermicularis*.

Ascaridia: Ascaridia comprise a group with many genera and species. They are characterized by three lips with lateral papillae. The superfamily Ascaridoidea, characterized by large worms, comprises the genera *Ascaris* and *Toxocara* that are important in pulmonary diseases.

Spirurida: Comprises the largest number of parasitic nematodes. Most require in their life cycle an arthropod intermediate host. The most important group in the Spirurida is the filarial worms, which belong to several genera including *Wuchereria*, *Brugia*, *Loa*, *Onchocerca*, *Dirofilaria*, and *Mansonella*.

Trichuroidea include *Trichinella*, *Trichuris*, and *Capillaria*.

5.2 Specific Pathogenic Organisms

5.2.1 Strongyloidiasis

5.2.1.1 Definition

Pulmonary strongyloidiasis is an infection of the lung caused by *Strongyloides stercoralis* (threadworm).

S. stercoralis has a worldwide distribution. Although high-risk groups are immigrants from developing countries or from southern, eastern, and central Europe, the disease can be found in the general population from other regions of the world and in the southern USA, the prevalence is estimated as 0.4–4.0 % [4, 5].

When infection occurs, the skin is penetrated by filariform larvae contracted from soil that is contaminated with larvae-containing feces. The larvae pass through the lungs into the alveolar

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spaces and cause petechial hemorrhages and an inflammatory reaction of polymorphonuclear leukocytes and macrophages [5–7]. This stage is usually asymptomatic. After molting, the larvae move up the trachea, are swallowed, and pass to the gastrointestinal tract, particularly the small intestine, where they mature into parthenogenic females and produce eggs. Rhabditiform larvae hatch from the eggs, migrate into the lumen of the intestine, and are excreted with feces. A small number of rhabditiform (noninfective) larvae molt to reach the filariform (infective) stage in the wall of the colon and perianal skin. Therefore, in contrast to other helminthic parasites, *S. stercoralis* can complete its life cycle entirely within the human host. This allows for the process of autoinfection, which may persist for decades within the host.

5.2.2 Hyperinfection Syndrome

A hyperinfection syndrome occurs when there is accelerated differentiation and endogenous reinfection [4]. In this setting, there is an increased number of filariform larvae in the intestine, and increased invasion of the intestine and migration to the lung. Hyperinfection syndrome occurs in clinical conditions of corticosteroid or cytotoxic drug administration, in alcoholic patients, or a malignancy. During this syndrome, hematogenous spread may occur and involve multiple organs including those usually not affected. The clinical presentation may be as acute respiratory distress syndrome [8, 9].

5.2.2.1 Pathologic Findings

In the lung, during the initial stages of infection, the filariform larvae invade the alveoli and cause petechial hemorrhages. This results from migration of filariform larvae through the capillaries or small blood vessels. Cavities and abscesses may develop. Adult worms rarely develop in the bronchial mucosa, and probably only during hyperinfection (Fig. 5.1). The finding of filariform larvae in the lung is a sign of hyperinfection. During this phase, massive pulmonary hemorrhage or a histologic pattern of diffuse alveolar damage may occur [4, 5].

The filariform larvae are 300–600 μm long and 10–20 μm wide. They appear basophilic and may be seen within alveolar spaces or bronchioles. The rhabditiform larvae are approximately 400 μm long and 10–20 μm wide and are rarely found in the lung.

5.2.2.2 Differential Diagnosis

The classic morphologic features of the parthenogenic females, larvae, and eggs of *Strongyloides* are characteristic. One has to consider other potential causes of pulmonary hemorrhage and abscesses when these are associated with the larval forms of *Strongyloides*. Although they could be confused with *Strongyloides*, it is extremely rare to find migrating larvae of *Ascaris lumbricoides* or hookworm in histologic sections from the lung.

5.2.3 Dirofilariasis

Pulmonary dirofilariasis is an infection of the lung caused by the filarial nematode, *Dirofilaria immitis*, the dog heartworm. Human pulmonary dirofilariasis is thought to be transmitted from dogs to humans by mosquitoes of the genera *Aedes*, *Culex*, *Myzorhynchus*, and *Anopheles*, which represent the intermediate host and vector. Dogs are the most common definitive hosts but cats, foxes, muskrats, wolves, otters, and sea lions may also be infected. Canine dirofilariasis is widespread throughout the USA; human dirofilariasis is distributed in all 50 states in the USA with southern states having the highest incidence those along the eastern, southeastern, and southern coasts. The disease is also reported in Europe, Asia, Australia, Central America, and South America.

The adult worms reside in the right ventricle of the definitive host. Circulating microfilariae are ingested by mosquitoes, where they develop into infective larvae over 2 weeks. These larvae are inoculated into a new host by a mosquito bite, where they develop over a 60- to 120-day period

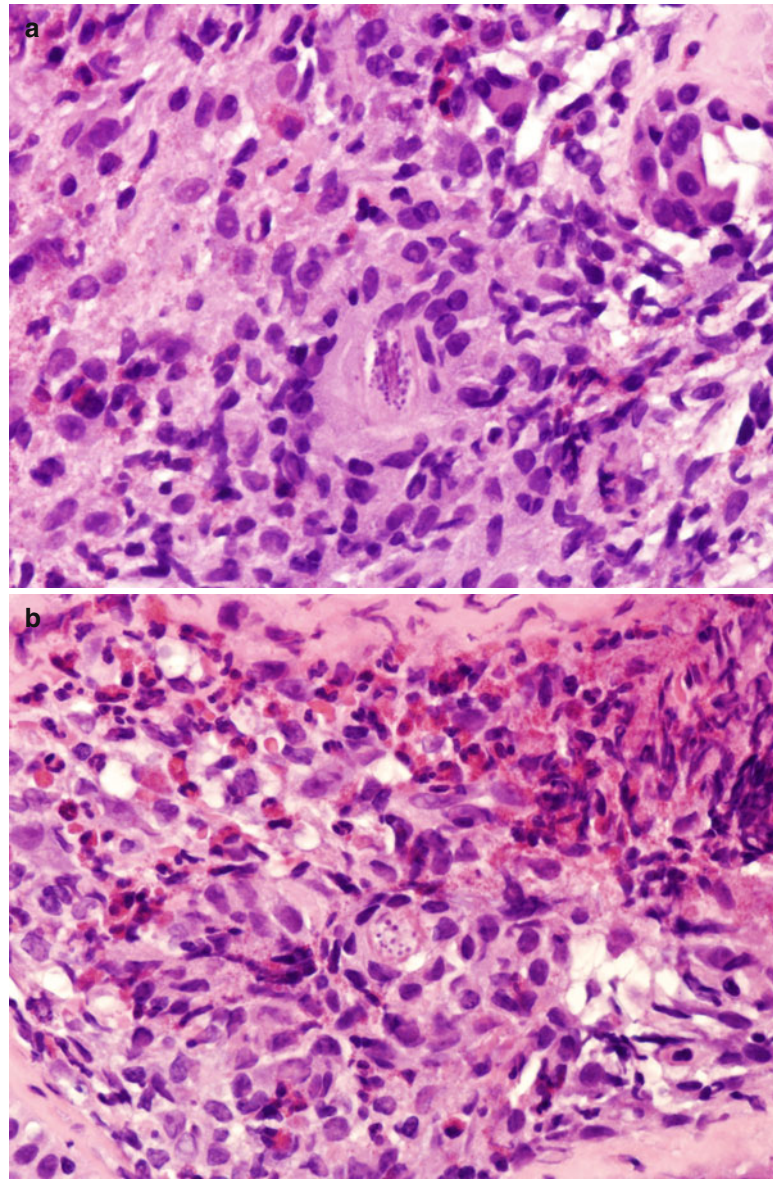

Fig. 5.1 (**a**) *Strongyloides* hyperinfection. A portion of the organism can be seen surrounded by a dense inflammatory infiltrate (20×). (**b**) Strongyloidosis in which severe tissue eosinophilia can be seen (20×)

into adult worms. When infected mosquitoes bite humans, the L3 stage larva is inoculated in the subcutaneous tissue where it develops to L4 stage larva. The L4 stage larva migrates to the muscles to develop to immature adult worm. The immature adult then migrates to the heart and pulmonary vessels for final maturation. Ultimately, the worms reach the right ventricle where they often cause right heart failure in the definitive host. Since humans are unsuitable hosts, the infective larvae usually develop no further and die. In patients who develop pulmonary dirofilariasis, the worm remains immature and reaches the right ventricle, where it dies and then embolizes to pulmonary arteries within the lung parenchyma. Humans are incidental host of this parasite. Lung is the most common infected organ in human. Infections of other organs such as conjunctiva, orbit, testicle, bladder, subcutaneous tissue, and peritoneal

cavity have also been reported [10–13]. When the *D. immitis* organisms die and embolize to the pulmonary vessels, they cause granuloma formation in the lung. The classic presentation is solitary lung nodules (see Chap. 1). Although most patients present with a solitary subpleural nodule, up to 10 % have multiple nodules. Approximately 75 % of nodules measure 2 cm or less, with a range from 1.0 to 4.5 cm. Most patients are asymptomatic. Some patients may present nonspecific symptoms such as chest pain, coughing, fever, hemoptysis, and dyspnea (see Chap. 1). Because the nodule may mimic a malignant tumor in imaging studies, surgery is usually performed and often frozen diagnosis is required. Macroscopically, the lung nodule is well-circumscribed. Dead *D. immitis* could be found within vessels on gross examination. Microscopically, the nodule presents as a necrotizing granuloma. The dead *D. immitis* can be identified within a necrotic vessel. The cross section of the parasite is large and measures about 200 μm. The characteristic microscopic feature is multilayered thick cuticle surrounding complicated internal organs. *D. immitis* pulmonary infection is a self-limiting process with no long-term complications. However, it is important to keep this entity in the differential diagnosis for solitary lung nodules. The nodules are usually stable without change in size over time. Occasionally, the lesions may mimic pneumonia and subsequently evolve into nodules over a period of weeks. Calcification is a rare and late complication.

5.2.3.1 Pathologic Findings

Grossly, dirofilariasis usually causes a single, circumscribed, rounded, subpleural nodule with a granular, yellow-gray cut surface.

Microscopically, the nodules consist of well-circumscribed round areas of necrosis surrounded by an inflammatory and fibrotic border (Fig. 5.2). In the center of the infarct, necrotic alveolar walls can often be seen. There is usually a mild to moderate inflammatory infiltrate at the edge of the lesion consisting of lymphocytes, plasma cells, and eosinophils. When the eosinophils are numerous, Charcot-Leyden crystals may be present. There may be granulomatous inflammation at the border, with palisading histiocytes and Langhans' multinucleated giant cells.

Sometimes, the dead worm can be identified within small- to medium-sized artery within an area of infarct (Fig. 5.3). The worm often appears

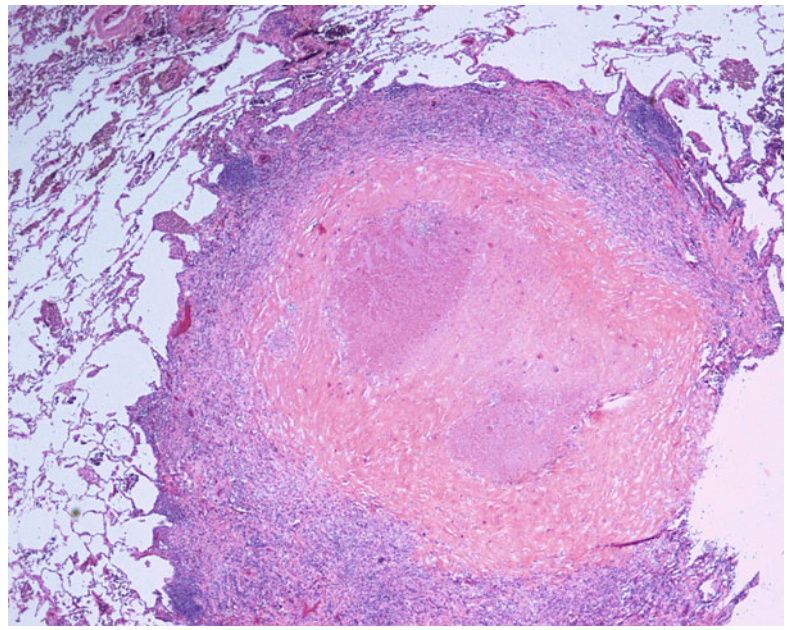

Fig. 5.2 Typical aspect of a nodule due to dirofilaria. There is a central area of necrosis in which in many cases it is possible to find the organism surrounded by fibrosis and inflammatory cells (5×)

fragmented and partly calcified. The blood vessel and worm may be more conspicuous when visualized in sections stained with the Movat pentachrome stain (Fig. 5.4). The worm may appear in multiple cross sections if it is coiled within an arteriole. The infected blood vessel may show vasculitis consisting of chronic inflammation and eosinophils. Vascular occlusion is not caused solely by the presence of the worm but also by the marked intimal fibrosis. The worm measures 125–300 μm in diameter. It has a smooth multilayered cuticle, 5–25 μm in thickness. There are prominent external transverse striations and internal longitudinal cuticular ridges. There is abundant somatic muscle and a centrally placed intestine. The number of reproductive tubes helps determine the sex of the worm since males have one and females have two, but this may be difficult to discern in necrotic worms and is not necessary to establish the diagnosis.

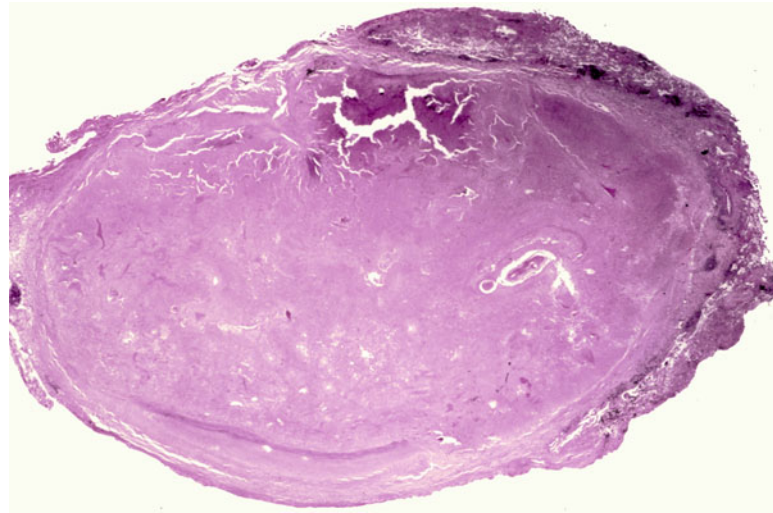

Fig. 5.3 *Dirofilaria* can be seen in vascular structures where they can cause thrombosis and secondary lung infarcts (2×)

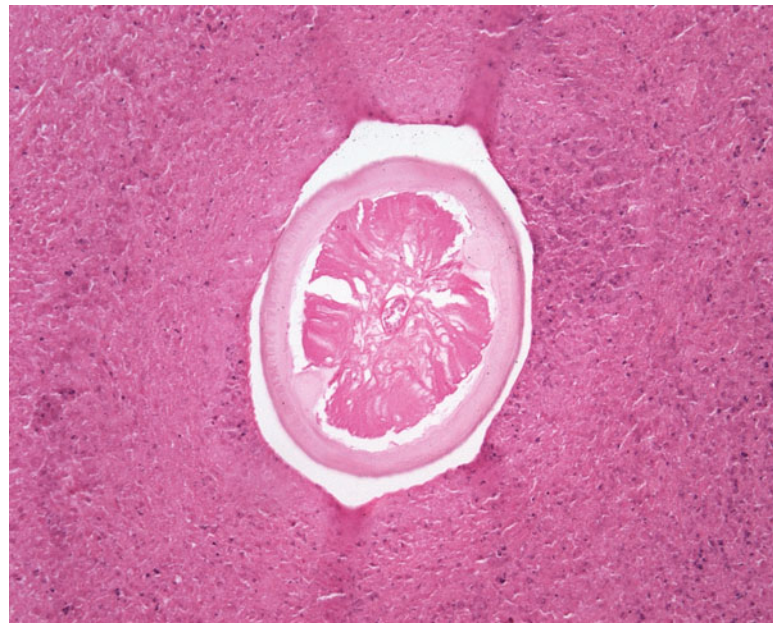

Fig. 5.4 Cross section of *Dirofilaria immitis* showing a thick triple-layered cuticle and two prominent lateral cords (100×)

5.2.3.2 Differential Diagnosis

Most dirofilarial lesions are initially mistaken for infarcts or necrotizing granulomas due to mycobacteria or fungi. Identification of the organism is best appreciated at low-power scanning examination and sometimes requires a search of multiple sections. The eosinophilic staining of the worm makes it difficult to appreciate since the blood vessel and surrounding necrotic tissue are also eosinophilic.

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The Trematodes

6

Abida K. Haque

6.1 Introduction

The trematodes belong to the phylum Platyhelminthes or flatworms. Their characteristics include a flattened body with an outer tegument and two suckers, which is a characteristic feature of this group. Most trematodes are hermaphroditic, with well-developed reproductive organs and digestive system. Trematodes inhabit the alimentary canal of vertebrates and may involve other organs such as liver, biliary tract, lung, and urinary tract. Trematode infections are prevalent worldwide [1–3].

There are three groups of trematodes: Monogenea, Aspidogastrea, and Digenea.

Monogenea are external parasites of fish, with direct life cycles.

Aspidogastrea are endoparasites with their entire ventral surface as the adhesive organ.

Digenea are also endoparasites with simple adhesive organs and require one or more intermediate hosts.

This chapter is focused on the Digenean trematodes. Most Digenean trematodes, including *Fasciola* species, *Clonorchis sinensis*, and *Schistosomes*, primarily parasitize the liver and intestines in the human host, with the exception of *Paragonimus westermani*, which infect the lungs. These parasites have a complex life cycle, involve a mollusk host, and may have up to six larval stages. The trematode eggs have a smooth hard shell, and majority of them are operculate.

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6.2 Specific Pathogenic Parasites

6.2.1 *Paragonimus westermani*

Paragonimus westermani is the commonest species of the genus *Paragonimus*, parasitizing both humans and animals, causing pulmonary paragonimiasis. There are 16 species of *Paragonimus* that are pathogenic to humans. The infection is seen in the Far East including China, Japan, Korea, Taiwan, Philippines, Indonesia, and parts of Southeast Asia including India and Nepal [4]. *Paragonimus* sp. infection has been also reported from Canada, North and Central America [5], and parts of South America. *Paragonimus africanus* has been reported from Nigeria, Libya, Liberia, and Zaire [1–3].

The adult fluke is egg-shaped, thick, fleshy, and red brown in color, measuring 7.5–20 mm long and 2.5–5 mm thick. The body wall has a thick tegument up to 40 um, two or more layers of smooth muscle, and a row of tegumental cells. There is a ventral and oral sucker and well-developed ovary, testes, uterus, vitellaria, and excretory bladder within the parenchyma. The eggs are golden brown, oval with thick birefringent shell, and 80–120 um by 45–65 um, with a flat operculum at one end (Figs. 6.1, 6.2, and 6.3).

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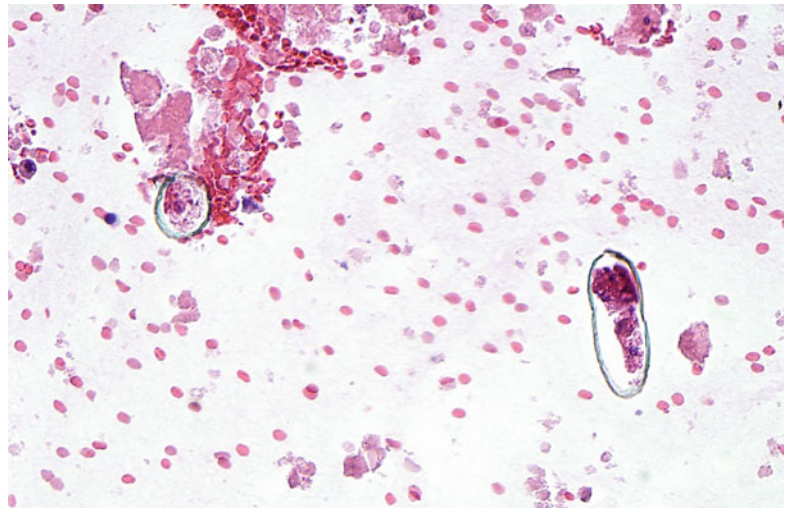

Fig. 6.1 Low magnification photomicrograph of eggs of *P. westermani* diagnosed in a fine needle aspiration biopsy, using cell block preparation. The eggs are surrounded by inflammatory cells and have thick shells (Photograph courtesy of Dr. Rodolfo Laucirica, Department of Pathology, Baylor College of Medicine, Houston, Texas)

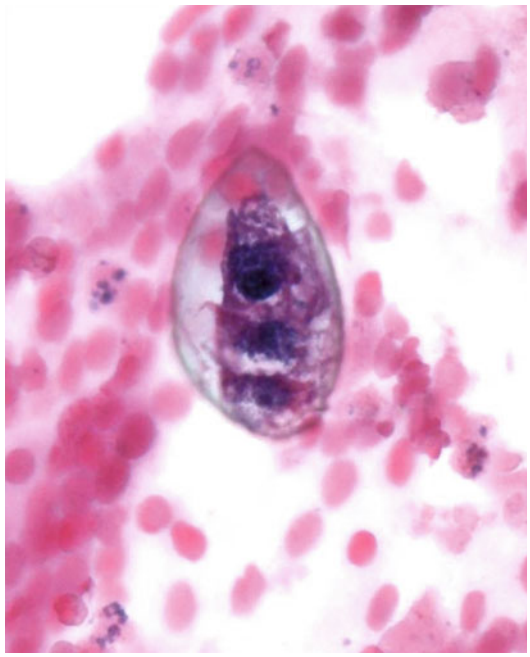

Fig. 6.2 Higher magnification of a *P. westermani* egg showing thick birefringent shell wall and a flattened operculum at one end (Photograph courtesy of Dr. Rodolfo Laucirica, Department of Pathology, Baylor College of Medicine, Houston, Texas)

The adult worms live in pairs or triplets in the respiratory tract of humans, encapsulated within a cyst. Eggs laid by the worms are either coughed up or swallowed and excreted in the feces. In the external environment, the eggs become embryonated, hatch in 2–3 weeks, releasing a ciliated miracidia, and seek the intermediate host, the snail. Within the snail, the miracidia develop into the cercariae, which invade the second intermediate host, a crustacean such as a crab or crayfish evolving into the metacercariae, the infective stage for mammalian host. Human infection occurs by eating inadequately cooked crab or crayfish. Human infection can also occur from ingesting raw or partially cooked meat of infected pigs and dogs. Once ingested, the metacercariae excyst in the duodenum, penetrate through the intestinal wall into the peritoneal cavity, and migrate through the diaphragm into the thoracic cavity and lungs, where they become encapsulated and develop into adults. Most adult worms die in 5–6 years, but some may live for up to 20 years. *P. westermani* requires encapsulation of two or three worms for insemination; therefore, when they are single, these worms migrate extensively in the thoracic cavity to find a mate, producing much inflammation. *P. pulmonalis* prevalent in Japan, Korea, and Taiwan is parthenogenetic and does not require another worm for insemination [1].

6.2.1.1 Clinical Manifestations

The clinical manifestations of paragonimiasis are nonspecific, and some patients may be asymptomatic. With heavy infection, patients develop cough, dyspnea, chest pain, fever and night

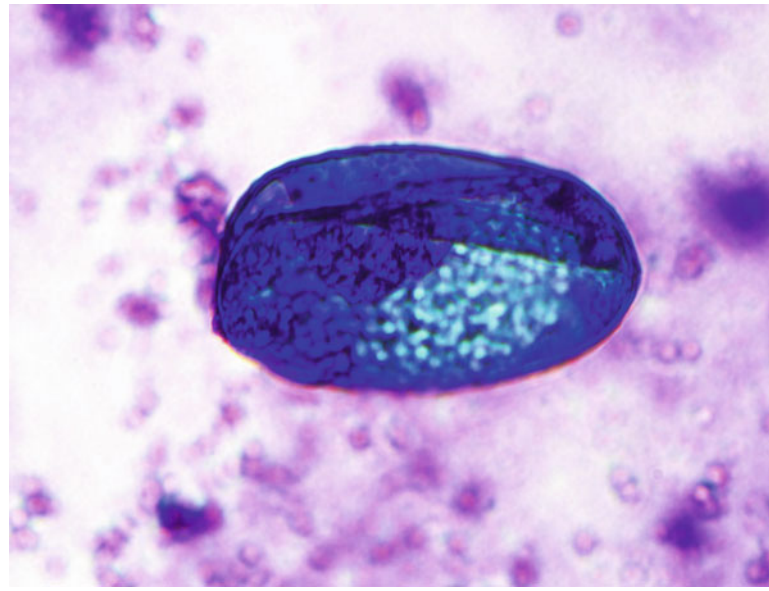

Fig. 6.3 *P. westermani* egg seen with Giemsa-stained cell block preparation of the fine needle aspirate of lung (Photograph courtesy of Dr. Rodolfo Laucirica, Department of Pathology, Baylor College of Medicine, Houston, Texas)

sweats, and hemoptysis. Bronchitis and bronchiectasis may develop over time. Peripheral eosinophilia of up to 25 % may be seen. Chest X-rays may show diffuse infiltrates, consolidation, nodules, pleural effusion, or empyema. The disease clinically resembles pulmonary tuberculosis. The active infection with nodules can be mistaken for malignancy on positron emission tomography with computed tomography scans (PET-CT) [6, 7]. There may be dissemination of infection to the brain, heart, abdominal cavity, and subcutaneous tissue, with patients presenting with unusual clinical manifestations [8].

6.2.1.2 Pathology

The pathologic changes depend on the degree of infestation, host immunity, and duration of infection. In the lungs, there is local suppurative inflammation surrounding the adult worm and eggs, with abundant plasma cells, neutrophils, eosinophils, macrophages, and foreign body giant cells. Older lesions may not show the parasites, but have a thick fibrous wall and chronic inflammatory infiltrate, and calcifications [1]. Since many worms lodge near the large bronchioles or bronchi, the inflammatory reaction is mostly around the airways, and the cysts may rupture and discharge eggs into the airways; there is often pleural inflammation, thickening, and fibrosis, associated with the invasion of the worms during migration. Fine needle aspiration (FNA) of lung lesions and pleural fluid or pleural biopsy may yield the characteristic eggs of *P. westermani*, surrounded by inflammatory cells. Figures 6.1, 6.2, and 6.3 show a cell block prepared from a lung FNA. The eggs of *P. westermani* have a flattened operculum at one end, a birefringent cell wall, and appeared to be embryonated.

6.2.1.3 Diagnosis

The suppurative inflammation and hemoptysis may be mistaken for bacterial pneumonia, pulmonary tuberculosis, and other parasitic infections, such as strongyloidiasis and dirofilariasis. Diagnosis depends on finding the characteristic ovoid, brownish, thick-shelled, and operculated eggs in the sputum, pleural fluid, and/or feces. Fine needle aspirate of the nodule may yield the eggs or parasite for a definitive diagnosis [9]. Serologic tests, particularly ELISA, are useful for diagnosis [10]. Recently, loop-mediated isothermal amplification (LAMP) assay has been used to provide a rapid and sensitive tool for detection of *P. westermani* DNA in sputum and pleural fluid [11].

6.2.2 Schistosomiasis

Schistosomiasis is infection of humans by trematodes belonging to the Schistosoma superfamily of trematodes. There are three main species pathogenic to humans, *S. mansoni*, *S. japonicum*, and *S. haematobium*, and three additional species that are less common with restricted geographic ranges. An estimated 200 million people in Africa, the Americas, and the Far East are infected with Schistosomes. Humans in these areas have been affected for many millennia; calcified ova have been found in the remains of Egyptian mummies. *S. mansoni* is the only species found in the Western Hemisphere, in Brazil, West Indies, and Puerto Rico. *S. japonicum* is found in Asia, including the Philippines and China. *S. haematobium* is found throughout most of Africa, southern Europe, and western Asia. In the USA, approximately 400,000 people are infected, primarily immigrants from other endemic countries [1, 12].

The Schistosomes are the only trematodes that live in the bloodstream of warm-blooded hosts. They differ from other trematodes in having separate sexes. The male worm resembles a rolled leaf and harbors the female in the ventral canal (the gynaecophoric canal). They require definitive and intermediate hosts to complete their life cycle [1]. Lung involvement is seen in infections with *S. mansoni*, *S. japonicum*, and *S. mekongi*.

6.2.2.1 Life Cycle

Adult worms of *S. mansoni* and *S. japonicum* live in the venous plexuses draining the rectum and colon, anterior mesenteric vessels, and portal vein, while those of *S. haematobium* live in the vesical plexus draining the urinary bladder. Since *S. haematobium* infection of the lung is not reported, we will focus on the other two *Schistosome* species in this chapter.

The eggs laid by the female worm enter the intestinal lumen from the vessels by a process of extrusion through the venule wall and pass in the feces. Once the eggs reach freshwater, they hatch, releasing a miracidium which enters a snail, and start asexual multiplication, with release of cercariae in the water. Humans are infected when the cercariae penetrate the skin, enter circulation to reach the portal vein in the liver, and mature into adult worms. The majority of adult worms live 2–4 years, but some may live longer. The adult males of the *S. mansoni* and *S. japonicum* are up to 15 mm, and females are up to 10 mm in length, and both have oral and ventral suckers. The adults of *S. japonicum* are longer and narrower. The eggs of *S. mansoni* are 114–175 um long and 45–68 um wide, yellowish brown, and have a lateral spine, with an acid-fast shell when stained with Ziehl-Neelsen stain. The eggs of *S. japonicum* are smaller measuring 55–85 um by 40–60 um and oval with a lateral spine.

6.2.2.2 Clinical Manifestations

Clinical manifestations of both *S. mansoni* and *S. japonicum* are related to the host reaction to the eggs, with formation of granulomas in the infected tissues. Heavy primary infection can cause high fever, hepatosplenomegaly, lymphadenopathy, eosinophilia, and dysentery (Katayama syndrome) and correlates with tissue migration of the worms. Chronic infection may be associated with diarrhea, weight loss, anemia, hepatosplenomegaly, portal hypertension, and ascites. Asymptomatic infections are common in most endemic areas [1, 12]. Pulmonary infection results from embolization of large number of eggs into the pulmonary vasculature with development of pulmonary hypertension, hemoptysis, cyanosis, and congestive heart failure. These complications occur in approximately 5 %, a small minority of schistosomiasis cases, and reportedly more common with *S. mansoni* infection [12]. The presence of hepatic infection and portal hypertension with portocaval collaterals is necessary for the development of pulmonary symptoms. Heavy infection of the lung may be associated with granulomatous pulmonary arteritis [13, 14].

6.2.2.3 Pathology

During active infection, granulomas at different stages of evolution may be seen in the colon, liver, and in the lungs. Early granulomas, both in the lung and in pulmonary arterioles, have many eosinophils, macrophages, and epithelioid cells,

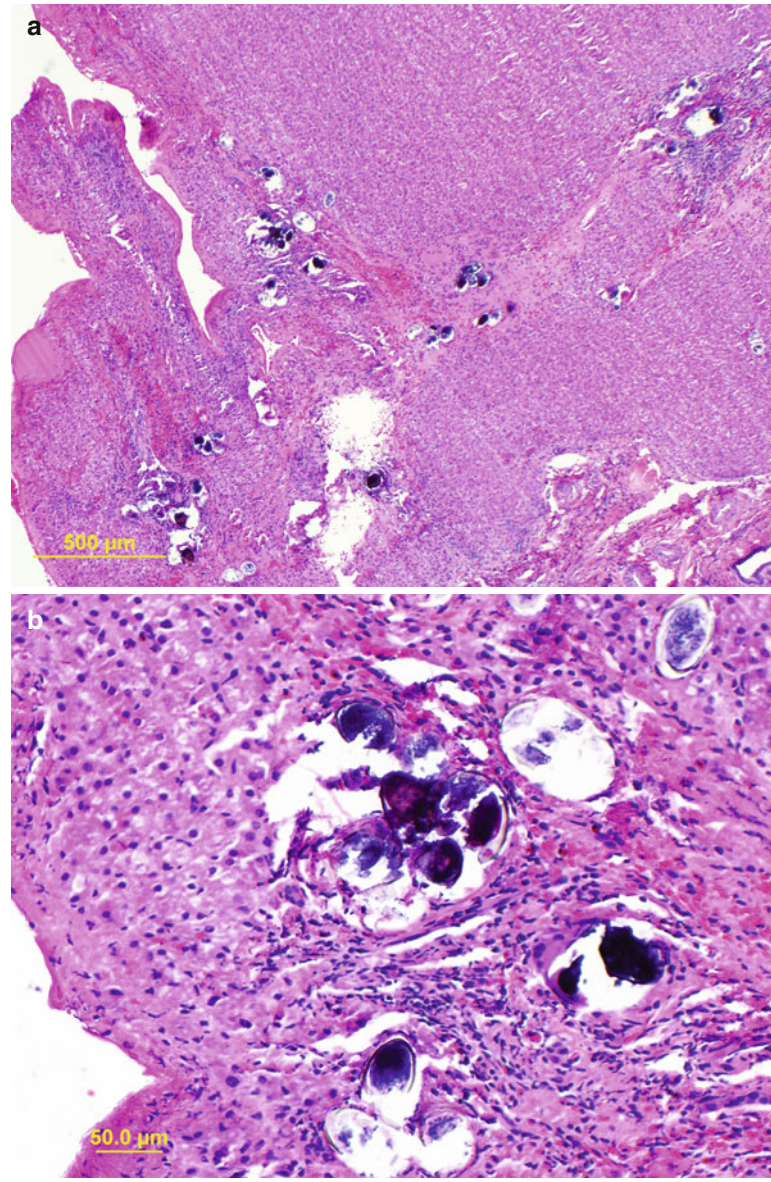

Fig. 6.4 Photomicrographs (**a**) (low magnification) and (**b**) (high magnification) of composite granulomas seen in the hepatic portal tract. The calcified eggs are surrounded by chronic inflammatory cells and fibrosis. Lung infections show similar histologic features (Photographs courtesy of Dr. Juan Olano, Department of Pathology, University of Texas Medical Branch, Galveston, Texas)

with the mature miracidium or the egg present in the center. On an average, the granulomas are 250–375 um in diameter. Pulmonary arterioles may show microthrombi, necrotizing arteriolitis, medial hypertrophy and hyalinization, and intimal thickening. Severe pulmonary hypertensive changes including plexiform lesions may develop [1]. *S. mansoni* granulomas are scattered and discrete, up to 550 um in diameter, and contain a single egg, while the *S. japonicum* granulomas form composite granulomas containing more than one or clusters of eggs in the center [1, 14–18].

Figure 6.4 shows multiple composite granulomas surrounding clusters of calcified eggs within liver portal tracts. There is fibrosis of adjacent hepatic tissue. Similar granulomas are seen in lung infections. The composite granulomas are seen in *S. haematobium* and *S. japonicum* infections.

6.2.2.4 Diagnosis

Diagnosis is based on the history of exposure and travel to endemic areas. Definitive diagnosis of schistosomiasis can be made by finding the characteristic eggs in the feces or urine, using concentration method if necessary. The eggs need to be measured to determine the species. When eggs cannot be found with the concentration method, a rectal biopsy may be required. Tissue diagnosis depends on finding the typical eggs or parasite. The eggs of *S. mansoni* and *S. japonicum* are acid-fast positive and those of *S. haematobium* are negative with modified Ziehl-Neelsen technique [1]. Serologic tests using ELISA can be diagnostic. A loop-mediated isothermal amplification (LAMP) assay may be used for detection of *S. japonicum* DNA in serum [19].

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Cestoda

7

Abida K. Haque

7.1 Introduction

Cestodes are metazoan organisms consisting of a chain of segments appearing as a ribbon, and commonly known as tapeworms. Most tapeworms parasitize the gastrointestinal tract of humans and animals. Most cestodes have life cycles that include at least one intermediate host. The eggs or the gravid proglottids containing the eggs are passed in the feces, to be picked up by the intermediate host, and their subsequent development into infective larvae. When humans ingest the eggs, larvae develop and often result in major health problems.

7.2 Specific Pathogenic Parasites

The classification of tapeworms is difficult because of the large number of species that exist and the lack of knowledge about the life cycle of many. A simple classification of the families that occur in the tissues of humans as larvae includes Diphyllobothriidae and Taeniidae. Taeniidae include the two genera Taenia and Echinococcus.

Most adult tapeworms measure from a few millimeters to 10 m and have a body consisting of a series of segments known as proglottids, a scolex, and a neck. The proglottids contain the uterus filled with eggs [1].

This chapter focuses on the genus Echinococcus.

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7.3 Echinococcus

The genus Echinococcus has four species: *E. granulosus*, *E. multilocularis*, *E. vogeli*, and *E. oligarthus*. In general, their life cycles are quite similar, although their geographic distribution and the disease they produce may be somewhat different. *E. granulosus* is reported from Europe, Asia, Australia, Africa, Canada, and the USA [2–4]. The incidence is relatively high in some areas, especially where sheep are bred and dogs are used for sheepherding. *E. multilocularis* is endemic in western Europe, Middle East, Japan, and China and also present in Canada and the USA. Dogs, foxes, and coyotes have been found infected with this species. Prevalence of the other two species, *E. vogeli* and *E. oligarthus*, is less common and seen in South America and Panama.

7.4 Morphology

The adult Echinococcus is small, 3–6 mm in length, with a scolex containing suckers and a double-crown hooklets. The size of the hooklets allows discrimination of the four species. There are only 4–5 proglottids, with 45–65 testes and many fertilized eggs in the terminal proglottid.

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The adult *E. multilocularis* is the smallest of the species, 2 mm in length and with only16–26 testes [1, 5].

7.5 Life Cycle

The adult tapeworms live in the small intestine of the definitive host, most commonly a dog or a cat. The eggs and proglottids are excreted in the feces and ingested by grazing animal such as the sheep, elk, pigs, camels, horses, or humans, which become the intermediate host. Once in the gastrointestinal tract, the eggs hatch and release the oncospheres, which penetrate the mucosa and disseminate to many tissues via the circulation. Hydatid cysts develop in the tissues of the animal hosts with formation of a central cavity and development of the germinal membrane and laminated layer. The cyst grows and starts forming protoscolex from the germinal membrane. The cycle continues when ingested by either a dog or other definitive host; the cysts grow to become mature tapeworms in their small intestine.

7.6 Clinicopathologic Features

In humans, majority of the hydatid cysts develop in the liver, followed by the lungs, kidneys, muscle, spleen, soft tissues, brain, and bone. Most of the symptoms are due to mechanical pressure from the growing cysts. Hepatic cysts are usually not palpable until 20 cm or so and may cause jaundice and portal hypertension.

Pulmonary hydatid *E. granulosus* cysts are a major public health problem in countries where dogs are used to care for large herds. Approximately 90 % of the pulmonary hydatid cysts are solitary, and 10 % are associated with a concomitant cyst in the liver [6]. Pulmonary cysts are asymptomatic in about one third of patients, and the rest may have pleuritic chest pain, dyspnea, or cough [7, 8]. If the cyst ruptures, copious cough with expectoration of the cyst contents may occur. Rupture into the pleural cavity results in pleuritis and shortness of breath. Lung involvement is higher in exposed children [9]. Younger patients also develop giant pulmonary cysts, >10 cm in diameter, and require surgical excision [10]. Rupture of the cyst may result in pneumothorax and empyema. Involvement of other organs produces symptoms based on the location and size of the cyst. Rupture of the cyst may cause hypersensitivity reaction. Urticaria, itching, and asthma may develop due to allergic reaction.

Although infection with *E. multilocularis* is less common than *E. granulosus*, it has a greater morbidity and mortality. *E. multilocularis* is a rapidly growing parasite with invasive characteristics, resulting in rapid dissemination and high mortality.

The infection is mostly limited to the arctic and temperate regions of the world, its definitive hosts are arctic foxes, and its intermediate host is small rodents. The infection in humans is accidental and most commonly involves the liver. The *E. multilocularis* cysts are slow growing, with destruction of the liver parenchyma and development of ascites and portal hypertension. The infection can metastasize to the brain, lungs, and mediastinum. The mortality with this infection is high and reported to be 67 % in the early studies [11]. It may be better now with earlier diagnosis and chemotherapy available.

7.7 Pathology

The hydatid cysts of *E. granulosus* are unilocular, spherical, and filled with clear fluid and may range from a few millimeters to many centimeters in diameter. The cyst wall has an external laminated layer and an internal germinal (embryonic) membrane and is surrounded by a thin fibrous tissue wall. The laminated layer is acellular and about 1 mm thick, whereas the germinal layer is delicate and semitransparent and 10–25 um thick. The cyst cavity may contain brood capsules, which arise from the germinal layer. Scolices arise from the inner germinal layer of the brood capsule. When the brood capsule detaches from the stalk, it becomes a free-floating daughter cyst and with the ruptured scolices forms the “hydatid sand” [5].

E. multilocularis infection results in polycystic and alveolar hydatidosis. *E. multilocularis* cysts are complex, multivesicular with infiltrative rather than expansile growth. The liver is the primary site of infection with *E. multilocularis*. The cystic lesion contains a semisolid gelatinous matrix and distorted germinal and laminated membranes surrounded by collagen with calcifications. No cysts are seen within the lesion; however, at the edge it may have small microcysts filled with clear fluid (Figs. 7.1, 7.2, and 7.3).

7.8 Diagnosis

Pathologic diagnosis is easy, when the characteristic laminated, amphophilic cyst wall is identified. Radiologic modalities including ultrasonogram,

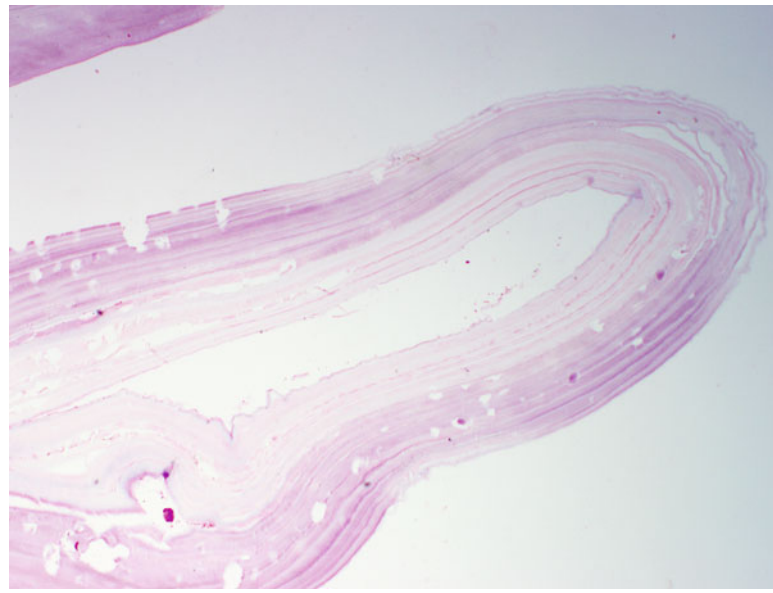

Fig. 7.1 Pulmonary hydatid cyst showing laminated membrane, without germinal layer and scolices

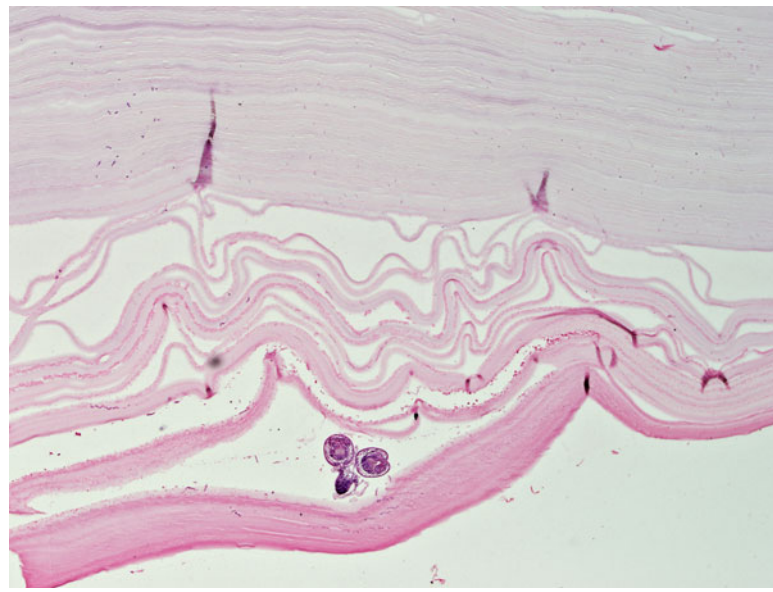

Fig. 7.2 A partially collapsed echinococcus cyst of lung with laminated membrane. The cyst cavity contains three scolices. No clear germinal layer is seen

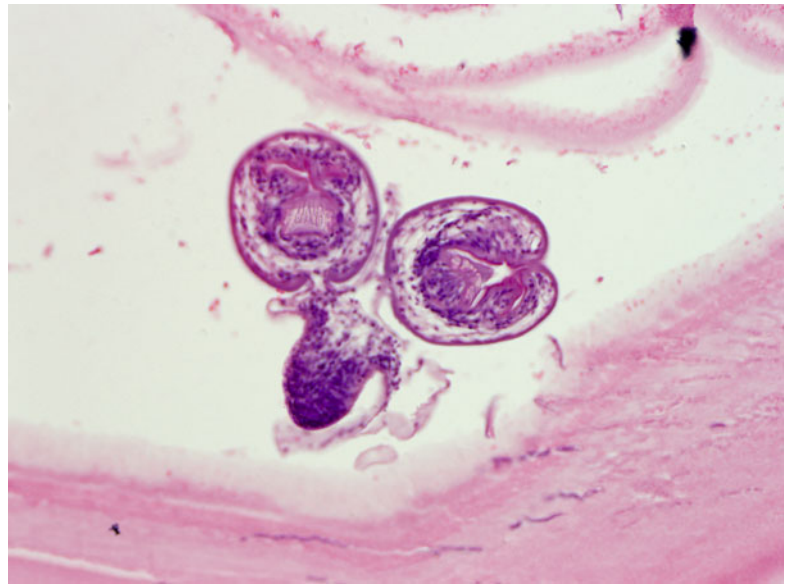

Fig. 7.3 Higher magnification of the scolices seen in Fig. 7.2

CT, or MRI can provide an accurate diagnosis in up to 97 % of cases [12]. However, if the cyst is ruptured, it cannot be differentiated from an abscess. In difficult cases, ultrasound or CT-guided needle aspiration of the cyst can yield the diagnosis, when the scolices or the hooks can be seen [13]. Serologic tests are less sensitive compared to most imaging techniques. Enzyme-linked immunodiffusion assays (ELISA) are the most sensitive of the usual serologic tests with up to 83.9 % sensitivity, and enzyme-linked immunotransfer blot (EIBT) has been reported to have 100 % sensitivity [14, 15].

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Arthropods and Bronchopulmonary Pathology

8

Rafael Martinez-Giron

8.1 Introduction

Members of phylum *Arthropoda* are the most numerous group of species in the animal kingdom. Arthropods are invertebrate, segmented, bilateral symmetrical animals, with articulated appendices and an exoskeleton composed by chitin. Depending on the group to which they belong, they have more or less complex life cycles, but in general, they all develop the following phases: egg, larval condition (with one or several instars) and adult stage.

The medical importance of arthropods is principally related to vector-borne diseases and by their capacity to introduce toxins and venoms through bites. Direct pulmonary diseases due to arthropods are uncommon in comparison to other parasitic diseases such as those due to protozoa and helminths, and this may be a reason why they not have not received attention in the medical literature and are, in most cases, neglected diseases [1–3].

Possible mechanisms through which the arthropods may cause bronchopulmonary pathology are (a) by inhalation or ingestion of larvae or adult forms, causing direct damage into airways and lung parenchyma or behaving as foreign bodies; (b) by the development of an allergic response; (c) by transmission of vector-borne diseases; and (d) by the direct introduction of toxins into the bloodstream. Table 8.1 shows the different types of arthropods that can be associated with bronchopulmonary pathology.

8.1.1 Mites

Lung infestation by mites (pulmonary acariasis) is an uncommon clinicopathologic condition in humans, being much more frequent in other animal species such as simians, rodents and birds.

Large series of human pulmonary acariasis have been reported in the English and Chinese literature (a total of 286 cases) (Table 8.2).

The presence of mites in respiratory cytology samples [4, 5] suggests that arthropod inhalation is a common route to acquire these organisms although luckily due to their size, not all mites are inhaled, and therefore, pulmonary acariasis is a rare disease. Mite species that may cause human lung pathology are shown in Table 8.3.

Different locations and environments such as house dust, mattresses and pillows, carpets, herbalists stores, greenhouses, bakeries, laboratory animals and farms are excellent habitats for mites that can potentially be inhaled and cause disease [6–8].

Clinical and anatomopathological descriptions of human pulmonary acariasis are scanty and very concise [9–11]. Symptoms such as cough, chest pain, hemoptysis and dyspnea are mentioned. Asthma and eosinophilic pneumonia have also been described. In a patient in which

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Table 8.1 Arthropods involved in bronchopulmonary pathology

Phylum: *Arthropoda*
Class: *Arachnida*
Order: *Astigmata* (mites)
Order: *Metastigmata* (ticks)
Order: *Scorpiones* (scorpions)
Class: *Hexapoda*
Order: *Diptera* (flies)
Class: *Insecta*
Order: *Blattaria* (cockroaches)
Order: *Coleoptera* (beetles)
Subphylum: *Crustacea*
Class: *Maxillopoda*
Subclass: *Pentastomida*
Genera: *Lingulata* and *Armillifer* (pentastomids)

Table 8.2 References on pulmonary acariasis in medical literature

Author/s	Year
Carter et al. (England)	1944
Soysa and Jayawardena (England)	1945
Carter and D'Abrera (England)	1946
Hall (England)	1946
Van der Saar (Dutch Islands)	1946
Wilson (Africa)	1947
Soysa (England)	1949
Deschiens (France)	1951
Soysa (Spain)	1952
De Figueroa (Spain)	1952
De Figueroa (Spain)	1954
Daniel et al. (Germany)	1955
Jorg (Argentina)	1956
Kijima (Japan)	1963
Akoun et al. (France)	1972
Li and Li (China)	1990
Chen et al. (China)	1990
Sun et al. (China)	1990
Xia et al. (China)	2005

lung resection was performed, multiple small yellow nodules were observed and, in the histological sections, bronchioles were distended with the epithelium destroyed and desquamated, containing many ova. The alveolar walls were also infiltrated by lymphocytes and histiocytes.

In contrast with few reports of human acariasis, macroscopic and histological findings of pulmonary acariasis in simians are numerous

Table 8.3 Mite species involved in pulmonary pathology

Family Acaridae
Acarus siro
Tyrophagus putrescentiae
Caloglyphus berlesei
Family Carpoglyphidae
Carpoglyphus lactis
Family Cheyletidae
Cheyletus eruditus
Family Echymyopodidae
Blomia tropicalis
Family Glycyphagidae
Glycyphagus domesticus
Lepidoglyphus destructor
Family Pyroglyphidae
Dermatophagoides farinae
Dermatophagoides pteronyssinus
Euroglyphus maynei
Family Tarsonemidae
Tarsonemus granarius
Tarsonemus floricolus

and have been well described [12–19]. There are several species of mites that parasitise the lungs of monkeys, being *Pneumosinus* sp. one of them (*P. simicola* is the most frequently reported). The morphologic findings consist grossly of numerous small whitish nodules, up to few millimetres, scattered throughout the lungs (approximately 50 % are found in the upper lobes); histologically, the mite bodies appear in small cavitary lesions surrounded by granulomatous reaction with an inflammatory cell infiltrate composed of neutrophils, eosinophils and plasma cells. There is fibrosis, epithelioid cells and foreign body-type giant cells around the mite bodies. Associated with lung mites, the deposition of brown granular pigment is also detected, being this finding a diagnostic feature. By means of microincineration technique, siliceous material has been demonstrated [20].

Since in the majority of patients with human pulmonary acariasis the presence of mites in sputum is a frequent finding, the recognition of these arthropods in sputum smears is important (Fig. 8.1), although its observation is not always possible. For this reason, we have developed an

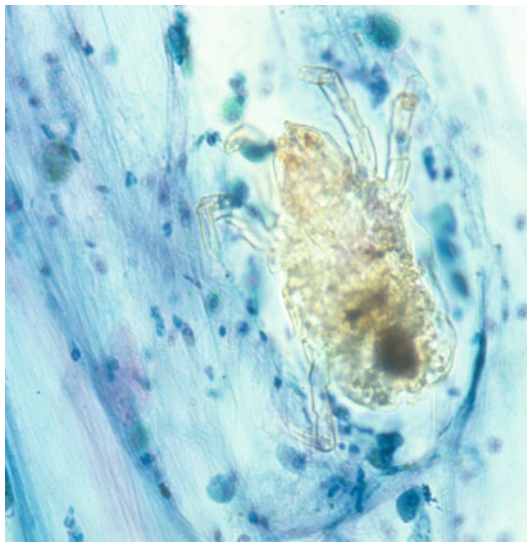

Fig. 8.1 House dust mite (*Dermatophagoides* sp.) in a sputum smear (Papanicolaou stain, ×200)

Fig. 8.2 House dust mite observed after bleach liquefaction to the sputum (×100)

experimental method [21] in order to recognise dust mites in sputum samples using commercial bleach as liquefying agent and observing the liquid material obtained under the microscope (Fig. 8.2).

On the basis of the presence of inhaled living mites in the distal airways an intriguing hypothesis is postulated that bronchial asthma is caused by the direct contact of these arthropods with the respiratory epithelium [22].

8.1.2 Flies

Invasion of tissues and body cavities by fly larvae is called myiasis. Human myiasis may be benign and asymptomatic or cause serious diseases. The disease may be acquired by inhalation and/or aspiration [23] where direct oviposition in cavities with poor hygiene takes place. In addition, nosocomial infestation in unconscious, immobile and debilitated patients has been also reported [24–27].

Bronchopulmonary disease has been associated with the following species and genus: *Alouattamyia baeri*, *Megaselia spicularis*, *Wohlfahrtia magnifica*, *Cuterebra* and *Gasterophilus* [28–32]. Nasal and pharyngeal involvement has been associated with other species such as *Oestrus ovis*, *Cochliomyia macellaria*, *Lucilia sericata* and *Sarcophaga* [33–37].

The most common symptoms are chronic cough, chest pain and occasional haemoptysis. Larvae may be expelled by means of cough [38, 39] or removal by endoscopy or thoracotomy. If larvae remain for some time in the respiratory tract, they may act as a foreign body (Fig. 8.3), with granulation tissue formation and chronic inflammatory cells surrounding the larva; occasionally focal necrosis may also be seen. In case of calcification, infarcted granulomas surrounding the larva may be observed.

If larvae can be obtained alive, it is possible to apply an entomological protocol on the basis of morphological characteristics (Fig. 8.4) that allows accurate identification by observing its development. This procedure is very useful in disciplines such as forensic entomology [40].

8.1.3 Pentastomes

Pentastomes are parasites that belong to a unique phylum with characteristics of both arthropods and annelids [41], although recent phylogenetic analysis results have confirmed that the pentastomids belong to the phylum Arthropoda [42]. Pentastomiasis (also known as porocephalosis) is the infestation by these parasites, being found mostly in tropical and subtropical areas.

Pentastomes are parasites which inhabit the respiratory tract of reptiles such as snakes,

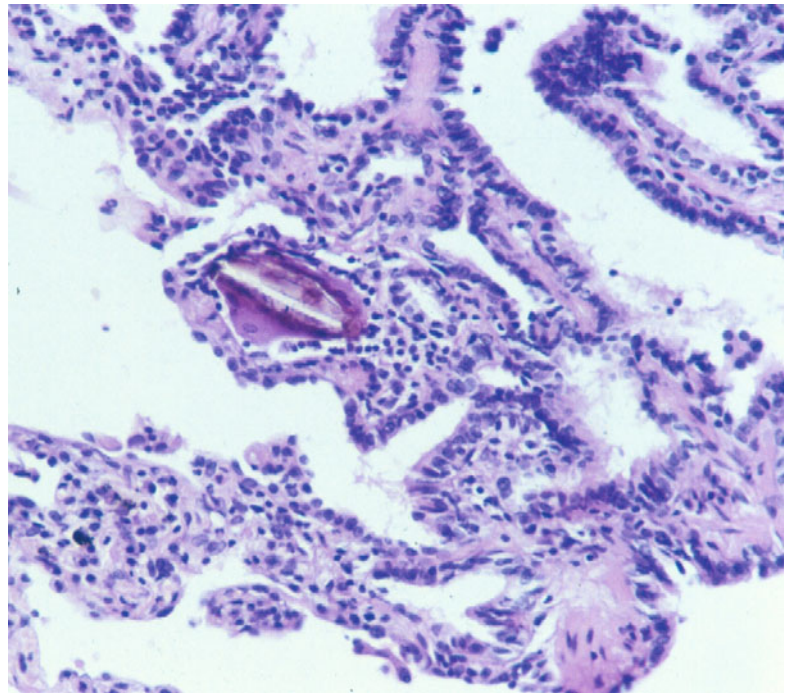

Fig. 8.3 The image shows the different stages (first-, second- and third-instar larvae and pupa) corresponding to the life cycle of the blowfly (*Calliphora vicina*)

Fig. 8.4 Lung biopsy. Focal chronic interstitial pneumonitis due to the presence of foreign body, tentatively interpreted as a larval structure (HxE, ×200)

lizards and crocodiles as the definitive hosts, being observed often as an incidental finding at autopsy of these animals (Fig. 8.5). Mammalian carnivores, including humans, may be intermediate hosts. Humans may acquire the infection by drinking water or eating food contaminated by an infected animal's faeces, by handling closely an infected animal (mainly big snakes) or by eating their raw and/or uncooked fleshes [43–45]. Thus, the zoonotic potential of these parasites, especially in tropical countries and with peculiar culinary habits, must be taken into account as a biological risk and an epidemiological factor [45–47].

Six species of pentastomids are known to infect humans but only two, *Armillifer armillatus* and *Linguatula serrata*, have been related to human pulmonary and nasopharyngeal pathology, respectively. Pulmonary infestation by both species is, in most of cases, an occasional finding observed in the course of a surgery, radiological or autopsy exam [48–51], although unspecific symptoms such as cough and night sweats have been related. Macroscopically, the visceral lesions are nodular or cyst-like. In order to demonstrate mature infective nymphs in tissue sections, both haematoxylin and eosin and trichomic stains offer good results. Pentastome morphology is very peculiar, with a series of typical features such as cylindrical or flattened in shape with external spiral rings, 4–6 mm body length (for *L. serrata*) and 13–23 mm body length (for *A. armillatus*), oral pairs of hooks, prominent spines attached to the parasite's cuticle (mainly in *L. serrata*) and striated muscle fascicles in the body cavity. Granulomatous reactions around encysted larva may be observed, with fibroblastic proliferation

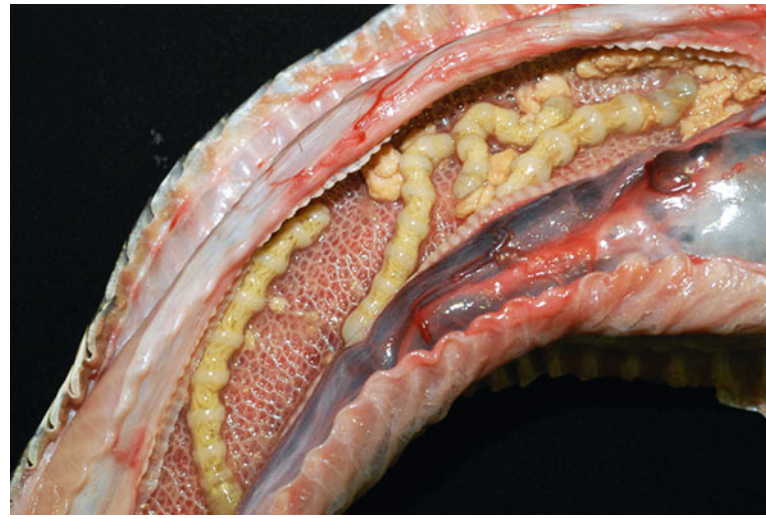

Fig. 8.5 Necropsied snake showing, in its respiratory tract, four pentastomids belonging to *Armillifer* sp. Kindly loaned by John Roberts, State of Alabama Dept of Agriculture and Industries

and chronic inflammatory infiltrate composed by lymphocytes, plasma cells and macrophages.

In case of nasopharyngeal involvement, the presence of parasites (*L. serrata*) in nasal discharge has been observed [52].

Other visceral affectations such us hepatic and peritoneal cavity, in the form of calcifications, tumour-like lesions, granulomas or miliary nodules, have been also described [50, 53–59].

Although nucleic acid sequences are available for certain species (*A. armillatus* and *P. crotali*) and tested in animals [60], these techniques are not conclusive diagnostic laboratory tests for pentastomiasis in humans, for what the histopathological exam continues being the gold standard for its diagnosis [61].

In the differential diagnosis of pulmonary pentastomiasis, other entities such as tuberculosis and parenchymal calcifications due to cysticercosis, paragonimiasis and myiasis must be taken into account [62]. Moreover, in case of the presence of vegetal matter in the lungs due to bronchoaspiration, vegetal tracheas (Fig. 8.6) may be misinterpreted as striate musculature to these parasites [63].

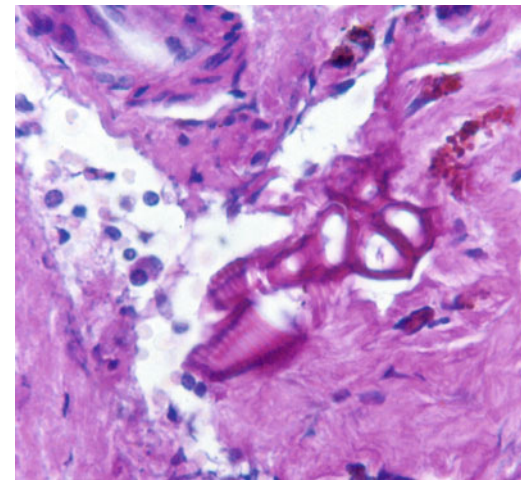

Fig. 8.6 Lung biopsy. Histological image shows vegetal tracheas in a case of pulmonary aspiration. Longitudinal (presence of rings) and transversal sections are observed (HxE, ×400)

8.1.4 Other Arthropods

Other biting and non-biting arthropods associated to pulmonary disease are ticks, scorpions, cockroaches and beetles.

Ticks have been reported as responsible for some diseases with pulmonary manifestations such as Lyme disease, ehrlichiosis, tularaemia and Rocky Mountain spotted fever (RMSF) [64]. Acute respiratory distress syndrome (ARDS) and respiratory failure are seen in Lyme disease, whereas pleural effusion and pneumonia are seen in patients with tularaemia and RMSF. ARDS has also been described in patients with *Borrelia* sp. infection acquired through the bite of an infected tick (*Ornithodoros* sp.) [65].

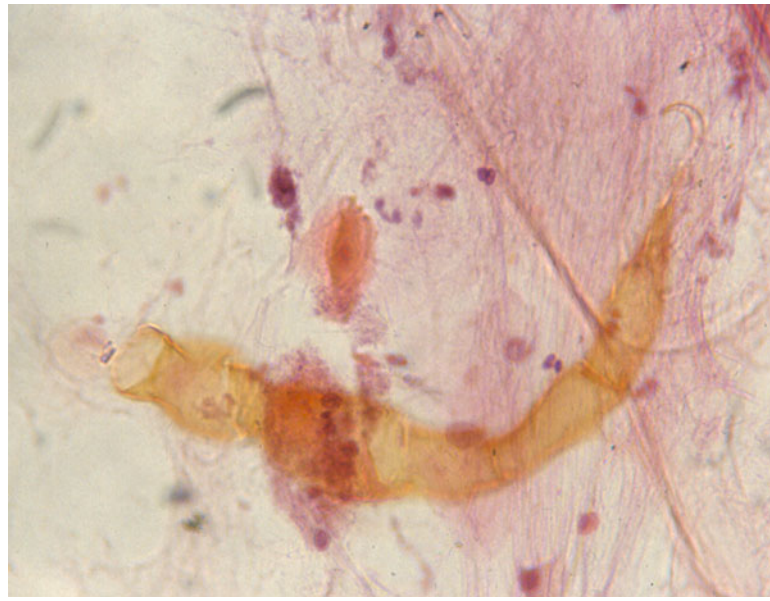

Fig. 8.7 Sputum smear. Distal part (complete tarsus) corresponding to an arthropod leg. It is possible to observe, at the end, the presence of a hook-shape nail (Papanicolaou stain, ×400)

Scorpion stings may cause severe respiratory failure which is most likely due to pulmonary oedema, myocardial dysfunction and secondary haemodynamic changes [66–69] since chest x-rays, high plasma protein levels and haemoglobin concentrations suggest this pathogenesis [70, 71]. Nevertheless, non-cardiac pulmonary oedema has also been described [72, 73] with ultrastructural studies that have shown injury to the alveolo-capillary membrane [74] and pulmonary infiltration with blood eosinophilia [75].

The clinical importance of cockroaches is not only due to their capacity as mechanical transmission of numerous microorganisms and as an important reservoir for infectious pathogens such as bacteria, fungus, protozoa and helminths [76] but also to their potential role as allergens and for the development of bronchial asthma [77, 78]. Mechanisms of induction of asthma are beyond the scope of this chapter.

Arthropods have also been found as foreign bodies in the respiratory tract [79]. There are some reports in the literature of cockroaches found as endobronchial foreign bodies [80–82]. All three patients reported were children, and in two cases, the diagnosis was delayed for several days.

There are few reports regarding beetles and pulmonary disease, but both entire adult specimens and larval hairs have been reported. In one case the arthropod penetrated to the tracheobronchial tree through a permanent stoma done in a patient who had undergone a laryngectomy for laryngeal squamous cell carcinoma. The beetle caused collapse of the patient's left lung [83]. In another case, carpet beetle larval hairs were found in a sputum cytology specimen [84] showing the typical "hastisetae" (hairs that cover the larva's body) in the shape of a barbed elongated structure with an arrow-shaped tip.

Carpet beetles in house dust have been considered as allergens and related to asthma [85].

Finally, it is possible to find in some cytology specimens, such as sputum smears, parts from arthropods (Fig. 8.7) and larval bodies (Fig. 8.8), which reflects the great inhalation capacity of the airways and airborne and freshwater contamination to the samples, the most probable causes for the presence of these foreign bodies in cytological smears [86, 87].

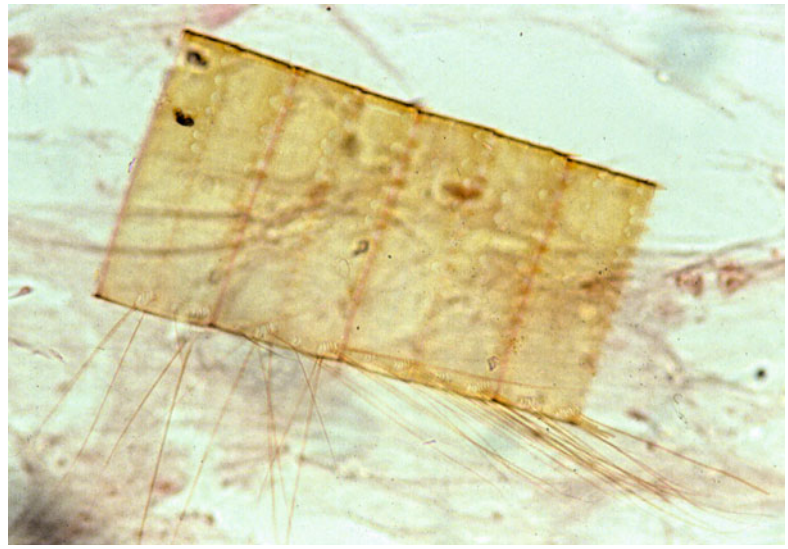

Fig. 8.8 Segments of a larva's body in a sputum smear. Thin hairs, in one of the edges, are observed (Papanicolaou stain, ×400)

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9 Interpretation of Lung Biopsies with Parasitic Versus Pseudoparasitic Structures: Artifacts That Resemble Parasites and Parasites That Resemble Artifacts

Roberto Barrios, Rafael Martinez-Giron, and Sergio Piña-Oviedo

The presence of uncommon or unexpected structures in sections may be a challenge to the general surgical pathologist, especially when the structures resemble a parasite. A good general knowledge of the morphology of common parasites allows the pathologist to distinguish parasites from structures that look like parasites. Structures that mimic parasites on sections may be the result of artifacts during preparation of slides or objects that are not related to the lesion or disease process. The presence of these "false parasites" may be *intrinsic* (as part of the tissue examined but not a parasite) or *extrinsic* (due to a number of objects in the environment that become in contact with the tissue or sections). It is uncommon to interpret normal histological structures as parasites, but tissues with various lesions including inflammation, hemorrhage, and calcification may produce structures resembling infectious agents. Specific chemical microenvironments, for instance, may cause precipitates that resemble parasites [1]. We can therefore consider the origin of pseudoparasitic structures as *endogenous* if the structures are produced by the organism or *exogenous* when the false parasite is incorporated in the tissue blocks or sections at some point during preparation.

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9.1 Endogenous Structures That Resemble Parasites in Tissue Sections

9.1.1 Tissue Components

9.1.1.1 Liesegang Bodies

Liesegang rings or Liesegang bodies are a rare finding that can be mistaken for parasites. They consist of laminated structures that are observed in cysts in the kidney, the breast, and occasionally in the lung (Fig. 9.1). They are formed by a chemical process. Raphael E. Liesegang, a German biochemist, described the chemical process in vitro in 1911. Liesegang bodies in tissue sections consist of concentric rings that form within supersaturated colloidal suspensions. The kidney, synovium, conjunctiva, and eyelid are also predisposed. Liesegang bodies vary from a few micrometers to several hundred micrometers in diameter. A single lesion may contain bodies of uniform or diverse diameters. Several publications mention that these structures have been confused with *Dioctophyma renale*, the giant kidney worm of carnivores [2–5]. The chemical and histopathological

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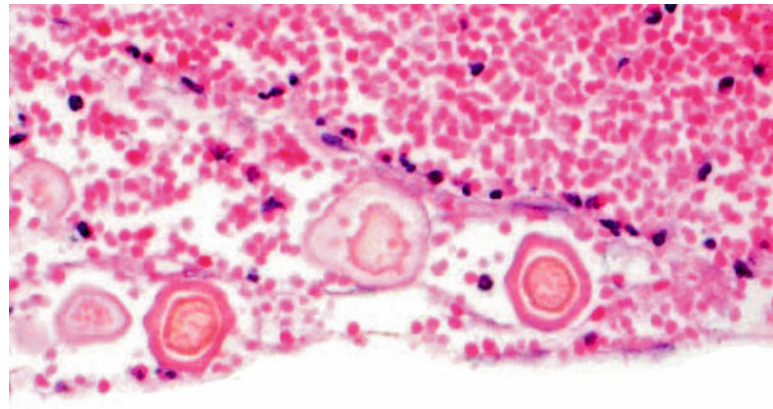

Fig. 9.1 Liesegang bodies. Hematoxylin and eosin stain

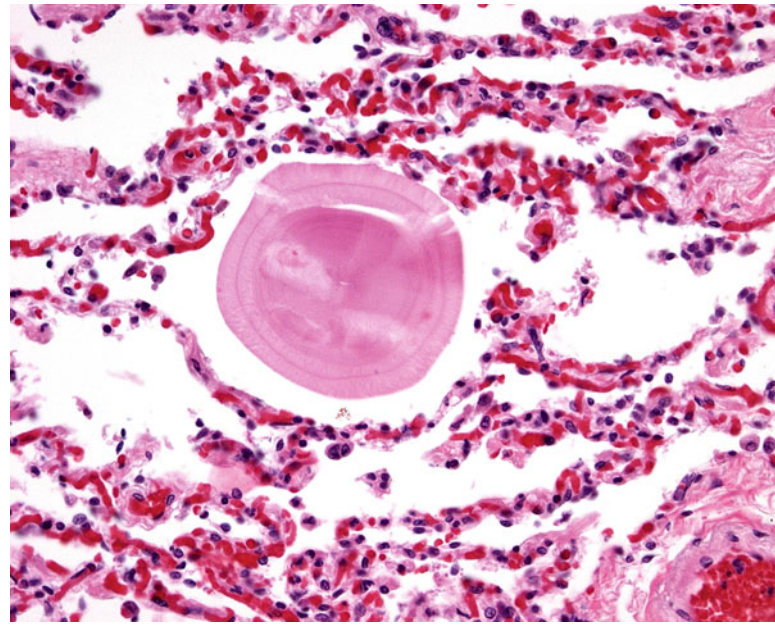

Fig. 9.2 Corpora amylacea. Hematoxylin and eosin stain

features of Liesegang bodies have been reviewed elsewhere and are beyond the scope of this chapter.

9.1.1.2 Corpora Amylacea

Corpora amylacea are small round to oval masses of unknown significance found in the pulmonary alveoli. They occur in the lungs of autopsy specimens with a frequency that varies between 0.6 and 2.8 %. They appear as eosinophilic bodies in hematoxylin- and eosin-stained sections and are composed of glycoprotein and made up of radiating fibrillar lines with delicate circumferential lines (Fig. 9.2). They are PAS positive, may contain a central core which may be positive for iron, and may be birefringent under polarized light (Fig. 9.3).

Although as far as we know, they are not clinically significant. Corpora amylacea may appear in large numbers and may be confused in specimens that are collapsed or show additional pathology [6].

9.1.1.3 Calcifications

Dystrophic and metastatic calcifications can occur frequently in the lung. They are usually not a diagnostic problem and their pathogenesis and morphology are covered in many

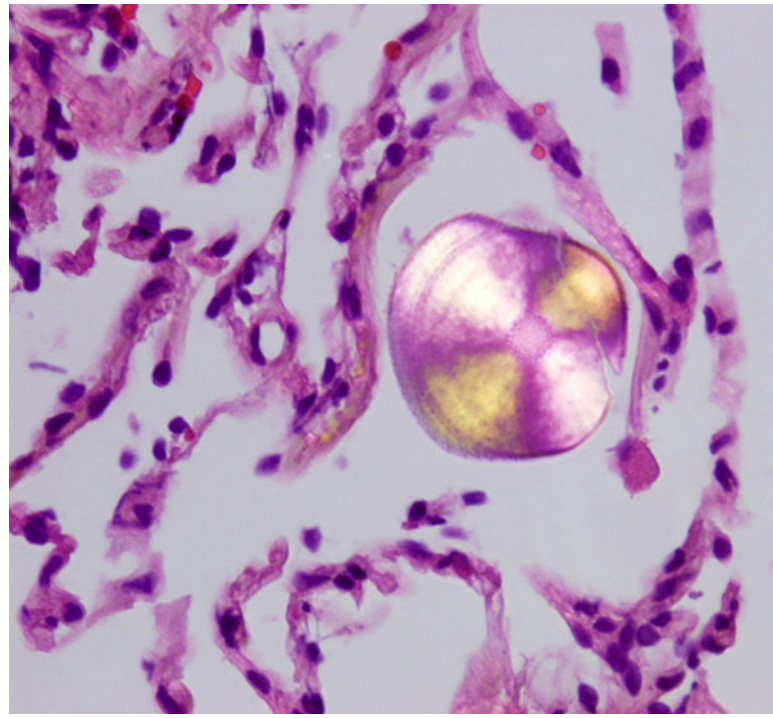

Fig. 9.3 Lung corpora amylacea usually show a Maltese cross-shaped birefringence under polarized light

textbooks. However, parasites can present as calcified structures [6, 7]. Parasites causing pentastomiasis (pentastomids or "tongue worms") can present as calcifications that may be interpreted as false parasites. There are three arachnid parasites whose larva stages cause pulmonary pentastomiasis: *Linguatula serrata*, *Armillifer armillatus*, and *Porocephalus moniliformis*. In pentastomiasis the chest x-ray shows numerous dense, discrete, crescentic or horseshoe-shaped opacities measuring about half a centimeter in diameter that represent a calcified nymph that adopts a semicircular posture when it dies. They can easily be mistaken for cysticerci but unlike cysticerci, they are only present in the lungs and not in the muscles. As discussed in other sections of this book, parenchymal and pleural calcifications also occur in *Paragonimus westermani* pulmonary infections and are most likely related to calcified mummification of the parasites. Pulmonary and thoracic lymph node calcifications are also seen in *Pneumocystis jiroveci* infections [8].

Calcified structures that may be interpreted as calcified parasitic forms include a rare disease, pulmonary alveolar microlithiasis, which is characterized by calcified round to oval "microliths" composed of calcium phosphate that can be found in bronchoalveolar lavages or present in transbronchial biopsies (Figs. 9.4 and 9.5). Pulmonary alveolar microlithiasis is a genetic disease with an autosomal recessive trait caused by mutation of the *SLC34A2* gene, which encodes a sodium phosphate co-transporter. The disease is found predominately in Japan, Turkey, Italy, and Mexico. Familial occurrence is present in 50 % of the cases. The microliths are basophilic and may show concentric "onion-like" rings (Fig. 9.5). They do not have a black center such as corpora amylacea [6].

9.2 Exogenous Structures That Resemble Parasites in Tissue Sections

9.2.1 Vegetable Particles

When structures from plants lodge in human tissue, they provoke inflammatory reactions and

may be confused with parasites (Figs. 9.6 and 9.7). Splinters in subcutaneous abscesses may resemble fly larvae or helminthes [9, 10].

Pulse pneumonia, also known as lentil pneumonia, is a disease of debilitated old people and the very young. Pulse pneumonia is caused by aspirated beans, peas, or lentils – most commonly from soup [11]. Pulse refers to the edible seeds of leguminous plants of these crops. Aspirated starch grains from these seeds may cause a granulomatous reaction, and the starch grains from the endosperm of the beans, peas, or lentils resemble nematode eggs or larvae [12].

Pollen grains in granulomatous lesions are often mistaken for helminthes eggs, especially

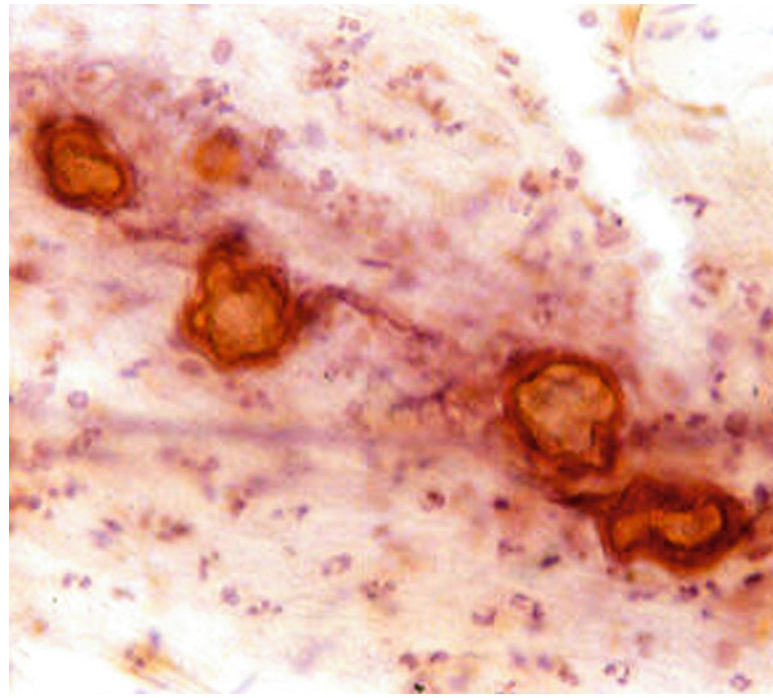

Fig. 9.4 Pulmonary alveolar microlithiasis. Bronchoalveolar lavage

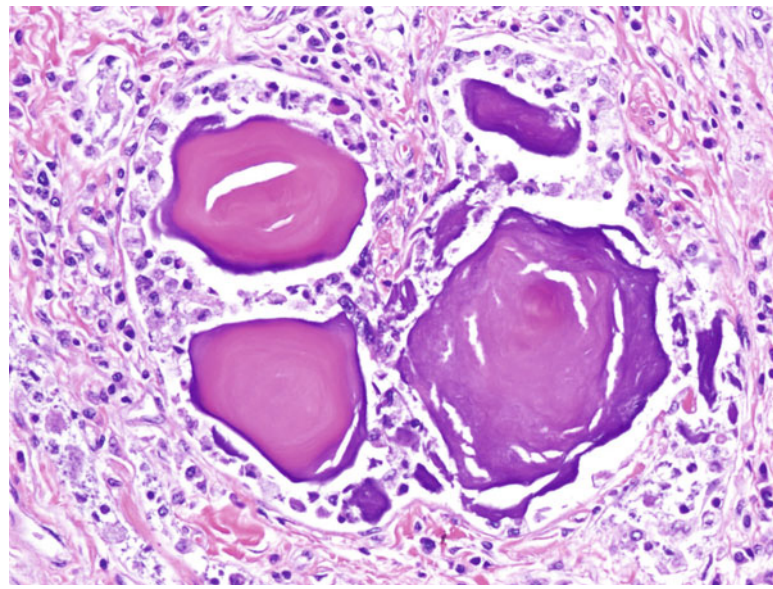

Fig. 9.5 Pulmonary alveolar microlithiasis. Lung biopsy. Hematoxylin and eosin stain

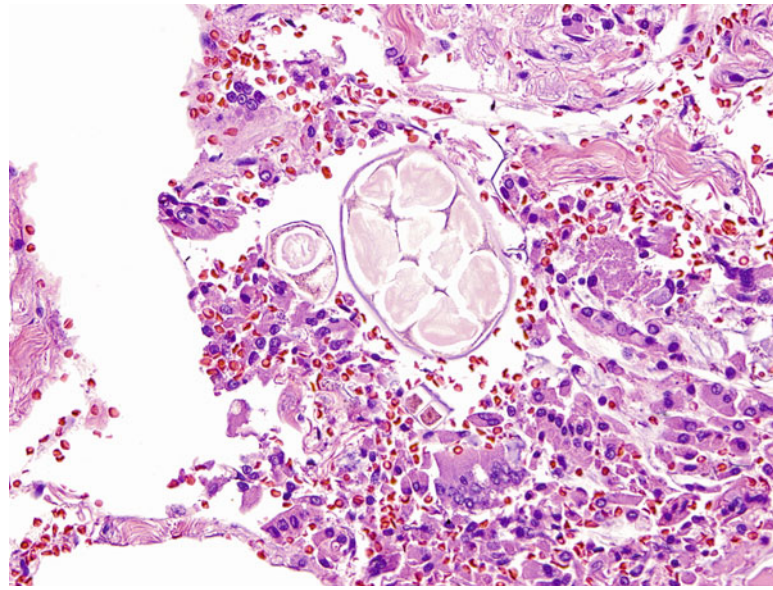

Fig. 9.6 Vegetable matter present in the alveolar spaces may mimic parasites. Lung biopsy in a case of aspiration pneumonia. Hematoxylin and eosin stain

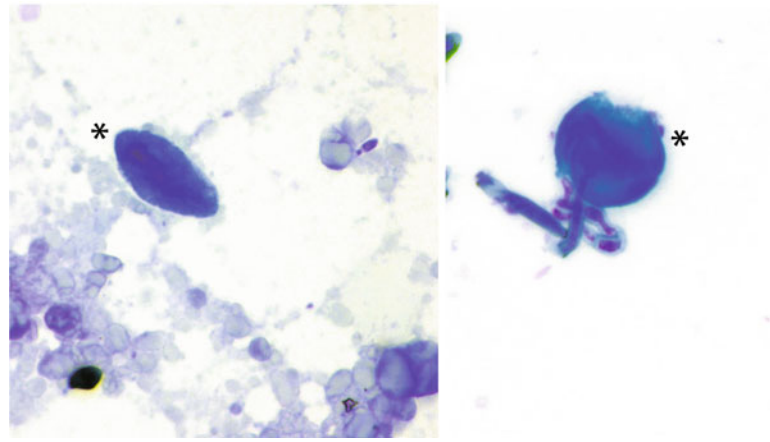

Fig. 9.7 Vegetable grains or seeds found in bronchoalveolar lavage may resemble ova of parasites (*). Giemsa stain

those of *Ascaris lumbricoides* or schistosomes. Spores of the clubmoss, *Lycopodium clavatum*, once used as lubricating powder on surgical gloves, cause granulomatous lesions and resemble eggs of *A. lumbricoides*. Pathologists may easily identify suspicious foreign bodies as plant material by staining with the periodic acid-Schiff (PAS) reagent. Plant material is brilliantly positive, whereas parasites generally are not. Microsporidians and ameba have PAS-positive features but not to the intensity where they would be mistaken for vegetable material.

9.2.2 Infectious Agents

Bacteria, fungi, and viruses that affect man may sometimes be mistaken for parasites. Cells infected with Cytomegalovirus (CMV) have been mistaken for *Strongyloides stercoralis* and *Toxoplasma gondii*. Likewise, these parasites have been mistaken as CMV, bacteria, fungi, and adult helminthes, and their eggs and cysts forms sometimes provoke surrounding rays or layers of antigen-antibody deposits, the so-called Splendore-Hoeppli phenomenon, which may be confused for cuticles of *Hypoderma* or nematodes.

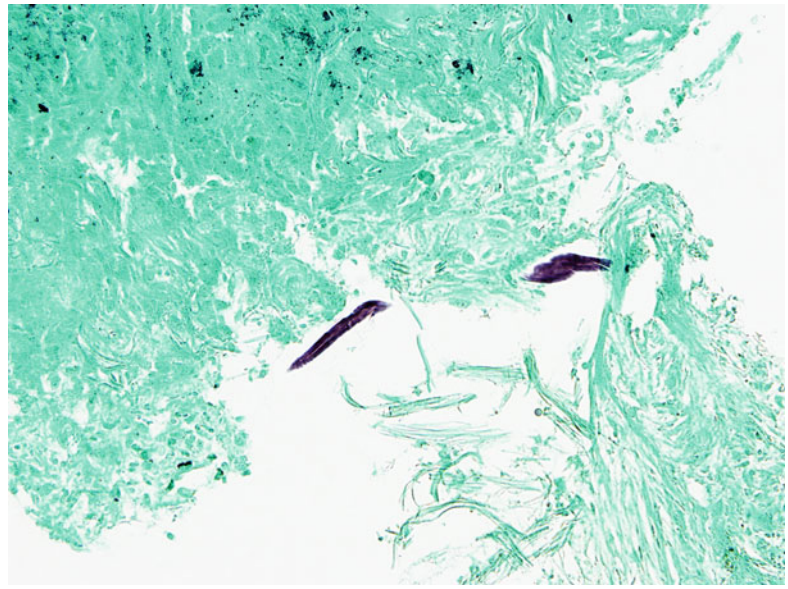

Fig. 9.8 Cotton fibers (*center*) can be positive for fungal stains. GMS stain

9.3 Staining Artifacts, "Floaters and Wipe Ons"

Staining artifacts are common, varied, and occasionally misinterpreted. An example is excessive and unevenly smeared egg albumin. The term "floater" describes elements that have contaminated a specimen during its processing. Floaters, which are confused commonly with pathogens, are bacteria or fungi picked up on the slide from the water bath or the hands of the laboratory personnel. Airborne or waterborne microbes may float onto the surfaces of tissue sections before the tissue is stained and coverslipped. Floaters occasionally create the illusion of etiologic association – a cluster of extraneous bacteria may settle into the center of a neutrophilic abscess and lead to an erroneous diagnosis. Careful focusing reveals that these microorganisms are above or below the plane of section and tend to have a random distribution. They have no constant relationship to the inflammatory lesions and tend to be on the slide away from the tissue section. Floaters are less commonly mistaken for invasive parasites. The airborne spore of the fungus *Helicosporium*, however, can resemble microfilariae.

The "wipe on" artifact usually comes from the cutting board, the dissection instruments, etc.

9.3.1 Miscellaneous

Cotton fibers may appear in surgical pathology specimens and simulate fungi or worms (Fig. 9.8). The spherical structures of myospherulosis, first identified in the subcutaneous tissues of East Africans, are now believed to be altered or mordant erythrocytes [13].

Infectious and noninfectious inflammations of lymph nodes sometimes contain tan, football-shaped structures called ceroid bodies, yellow-brown bodies, or Hamazaki-Wesenberg bodies. These bodies are seen in macrophages and in the stroma of lymph nodes and free in the sinuses; they may be overlooked because they are small and especially if the slides are overstained with eosin. They are derived from phagolysosomes, are common in sarcoidosis, and are not infectious agents [14, 15]. They have a natural brown color, are acid fast, and stain with PAS and Giemsa. Their importance is that they are sometimes mistaken for infectious agents, especially fungi (budding yeasts).

Patients who have had indwelling catheters or undergone endovascular procedures sometimes show fragments of catheter in the arterial lumen, surrounded by tissue reaction (Figs. 9.9 and 9.10). Sometimes, the plexiform lesions seen in

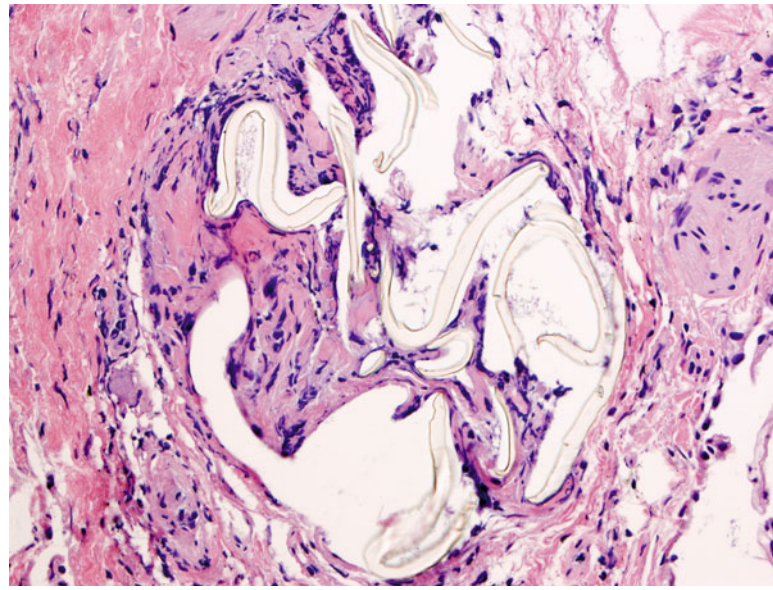

Fig. 9.9 Fragments of catheter inside the lumen of a distorted vessel can mimic larvae or parasites. Hematoxylin and eosin stain

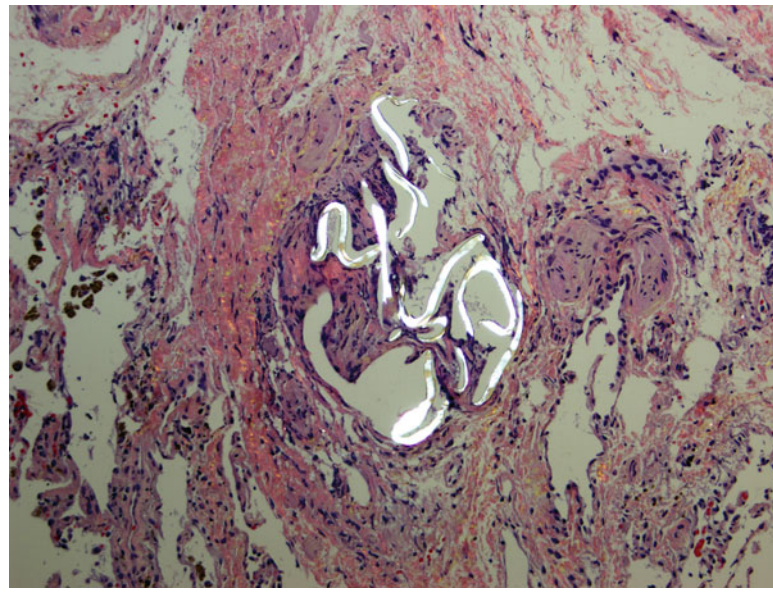

Fig. 9.10 The catheter material is strongly birefringent under polarized light

patients with pulmonary hypertension may be also mistaken for parasites (Fig. 9.11).

Embolized microcrystalline cellulose (MCC) and crospovidone may be found in lung biopsies or lung resections and also confused with parasitic structures. Both materials are used as excipients in pharmaceutical tables, and occasionally, individuals may inject dissolved pulverized tablets intravenously with embolization of these substances into the lung microvasculature. MCC is a matchstick-like and birefringent crystal that may resemble larvae or eggs. Crospovidone is deeply basophilic and coral-like and may suggest a calcified parasite (Figs. 9.12 and 9.13). MCC may be weakly positive with PAS, whereas crospovidone is negative. Presence of these foreign materials may elicit a foreign body giant cell

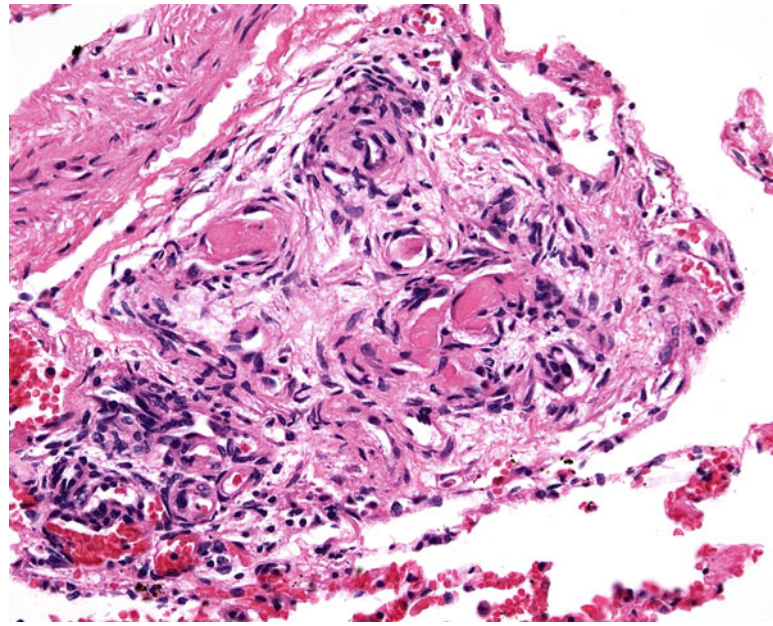

Fig. 9.11 Plexiform lesion in a patient with pulmonary hypertension. The convoluted vessels and eosinophilic material may be confused with parasites. Hematoxylin and eosin stain

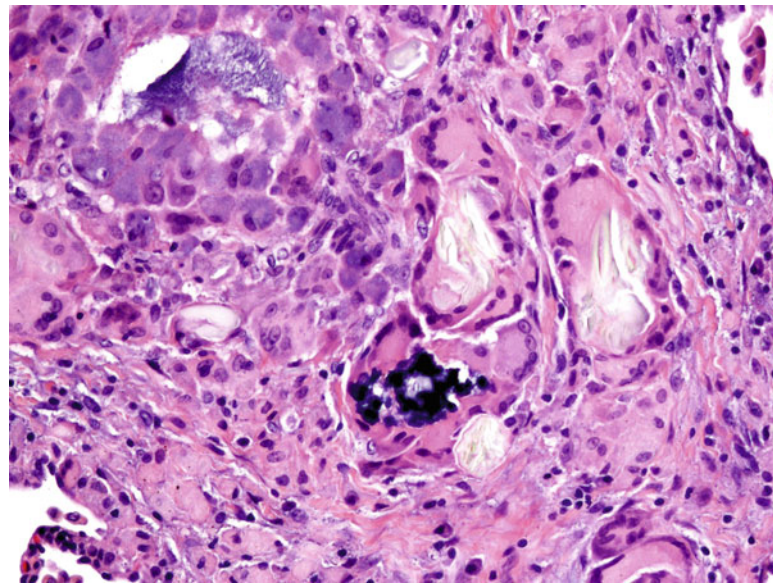

Fig. 9.12 Embolized MCC and crospovidone. MCC is refractile and may resemble parasite eggs or larvae. Crospovidone is coral-like and basophilic and may be confused with a calcified parasite. Both foreign substances are seen inside multinucleated giant cells. Hematoxylin and eosin stain

reaction that may be confused with a granulomatous reaction to an infectious agent [16].

9.3.1.1 How to Avoid the Deception of False Parasites

1. Many structures in tissue sections confused for worms are not vermiform. Examine serial sections to appreciate the geometry of the suspicious object.
2. Measure the maximum diameter of the foreign body. Is it consistent with the suspected parasite?
3. Take advantage of special stains (e.g., PAS for plant material) and polarized light microscopy (corpora amylacea, calcifications, MCC).
4. Do not expect a parasite just because the patient has traveled widely or describes exotic behavior.

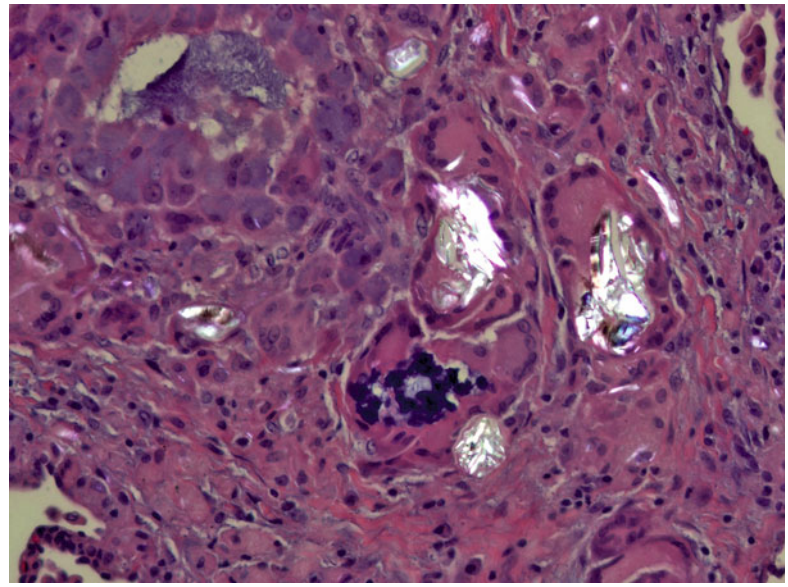

Fig. 9.13 MCC is strongly birefringent, whereas crospovidone is non-birefringent

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