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## *Chapter 1*

# ***ANGIOSTRONGYLUS CANTONENSIS: MORPHOLOGY, GENETIC VARIABILITY, ECOLOGY AND PARASITE-HOST RELATIONSHIP***

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## ABSTRACT

The nematode *Angiostrongylus cantonensis*, also known as rat lungworm, is the etiological agent of human angiostrongyliasis, which in turn causes eosinophilic meningitis. The synanthropic rodents *Rattus norvegicus* and *R. rattus* are the main definitive hosts in the Americas and molluscs are the intermediate hosts. Humans become infected accidentally by eating raw or undercooked snails or food contaminated with the infective L<sub>3</sub> larvae. This parasite is endemic in Asia and more recently in the Americas. This chapter reviews the parasite morphology, genetic variability, transmission dynamics in both intermediate and definitive hosts and parasite-host relationship, emphasizing the metabolic and physiologic alterations in hosts.

## INTRODUCTION

The nematode *Angiostrongylus cantonensis*, commonly known as the rat lungworm, is the etiologic agent of angiostrongyliasis, recognized as causing eosinophilic meningitis in humans and animals (Wang et al. 2008, 621; Reece et al. 2013, 477). The rats *Rattus norvegicus* and *R. rattus* are the main definitive natural hosts (Wang et al. 2008, 626; Maldonado et al. 2012, 310), mainly in the Americas (Simões et al. 2011, 1331; Moreira et al. 2013, 91), and snails and slugs act as the intermediate hosts (Maldonado et al. 2012, 309; Thiengo et al. 2010, 195). Land crabs, frogs, lizards and flatworms can also be involved in the life cycle as paratenic hosts (Wang et al. 2008, 621).

Adult worms are found parasitizing the pulmonary arteries of the definitive hosts where the mature female worms lay eggs, which developed into first stage larvae (L<sub>1</sub>). The L<sub>1</sub> move through bronchioles, alveoli and trachea in respiratory secretions, then are swallowed and excreted in rat feces (Maldonado et al. 2012, 308). The intermediate hosts acquire the capacity to transmit the parasite after ingesting the L<sub>1</sub> larvae, which need to perform two molts until reaching the infective form (L<sub>3</sub>). The life cycle is completed when the definitive host ingests an infected intermediate or paratenic host. Humans became infected after eating the intermediate or

paratenic hosts, and the L<sub>3</sub> larvae migrate via the bloodstream to the brain, provoking meningitis inflammation. In the natural definitive hosts, the L<sub>3</sub> are carried to the brain in sub-adult form and move again via the bloodstream to the pulmonary arteries (Maldonado et al. 2012, 308; Reece et al. 2013, 477).

This emerging neurologic disease has been reported extensively in Asia (Wang et al. 2008, 624-625) and more recently in the Americas (Morassutti et al. 2014, 399). This chapter reviews the parasite morphology, genetic variability, transmission dynamics in both intermediate and definitive hosts and characterizes the parasite-host relationship, emphasizing the metabolic and physiologic alterations in the definitive and intermediate hosts.

## MORPHOLOGY

*Angiostrongylus cantonensis* belongs to Metastrongyloidea superfamily, which is represented by four families, among them the family Angiostrongylidae (Anderson 1978, 1-40). This family is composed of the genera *Angiostrongylus* (Kamensky, 1905) and *Heterostrongylus* (Molin, 1861). Characteristics of the genus *Angiostrongylus* were reviewed by Spratt (2015). This genus is characterized by having a cephalic extremity without elevation, males with reduced caudal bursa with dorsal ray often with tiny terminal branches, externolateral ray well developed and arising from a common lateral trunk, ventral ray arising from a common trunk; gubernaculum small or absent, spicules long or short, vulva and anus near the terminal, female tail rounded and containing lung and blood vessels (Vicent et al. 1997, 44; Anderson et al. 2009, 178). *Angiostrongylus* spp. present six cephalic papillae, bucal capsule absent. Males with copulatory caudal bursa distinguished from the body with ventral rays divided at the extremity; externolateral ray shorter and diverging from other lateral rays, which fuse proximally; externodorsal ray arising separately from the dorsal trunk. Females have short and rounded caudal tip, oviparous, and vulva situated next to anus.

Based on the classification key of Anderson (1978), *Angiostrongylus* is divided into two subgenera, *Angiostrongylus Parastrongylus* and *Angiostrongylus Angiostrongylus*, differentiated by the origin of the externolateral ray, which is joined in a common trunk in the first and separated from the other lateral rays in the latter (Drózd, 1970). In other words, the externolateral ray is separate from the other two lateral rays, and in *A. Angiostrongylus* the externolateral ray is joined to a common trunk with the other lateral rays (Spratt 2015, 179). Twenty-one species of *Angiostrongylus* are known. These occur naturally in rodents, tupaiids, mephitids, mustelids, procyonids, felids, canids, and accidentally in avian, marsupial and eutherian hosts, including humans (Spratt 2015, 180-181). However, among these, *A. cantonensis* and *A. costaricensis* are the most studied species because of their wide distribution and medical importance. *A. cantonensis* was described in 1935 by Chen infecting *R. norvegicus* in China, and its morphology was subsequently redescribed when it was found parasitizing *R. norvegicus* and *R. rattus* and the intermediate host *Achatina fulica* in Brazil (Maldonado et al. 2010, 939; Moreira et al. 2013, 91-94; Monte et al. 2014, 1058-1060).

The morphology of the larvae (L<sub>3</sub>) of *A. cantonensis* found in snails present filiform body, cuticle with transversal striation and anterior extremity rounded, genital primordium characterized by a small ellipsoid structure in the second half of the body (features that do not aid in sex differentiation), buccal capsule long, esophagus long with filariform bulb, excretory pore and nerve ring situated in middle region of esophagus. The posterior extremity of L<sub>3</sub> is curved dorso-ventrally and the tail tip has a marked retreat in the dorsal surface (Moreira et al. 2013, 94).

The nematode *A. cantonensis* has been reported in different states of Brazil and its morphology has been reviewed. In 2010, *A. cantonensis* was isolated from *Achatina fulica* in two Brazilian states: Rio de Janeiro (Barra do Piraí and São Gonçalo municipalities) and Santa Catarina (Joinville). The adult worms from each isolate were morphologically and morphometrically compared with *A. cantonensis* from Pernambuco state, Brazil and Akita, Japan. The bursal rays of the São Gonçalo specimens were longer and thinner than the specimens from Japan and Pernambuco state. The size of

the spicules was very similar among the three isolated specimens. The bursal morphological characteristics of the Barra do Pirai specimens were more closely matched with specimens from Canton (China) (Chen 1935) and Pernambuco state (Thiengo et al. 2010, 197), whereas the São Gonçalo specimens were closely matched with *A. cantonensis* from Africa. Only minor morphological differences were observed concerning intra-specific variation (Maldonado et al. 2010, 940).

The worm *A. cantonensis* from Pernambuco state, Brazil showed large spicule size (greater than those observed in other species of *Angiostrongylus*) and the pattern of the bursal rays agreed with the original description by Chen (1935). Furthermore, the morphology of the Pernambuco isolate was similar to the Japan isolate. Comparative morphometric data of *A. cantonensis* adults found in Brazil is shown in Table 1.

In addition, the morphology of the larval stage was determined by scanning electron microscopy of *A. cantonensis* adults from from *A. fulica*. Scanning electron microscopy images of *A. cantonensis* specimens found in the Amazon region revealed ultrastructural characteristics similar to the descriptions reported previously by Lian-Yin et al. (1984) and Hüttemann et al. (2007).

Recently, Monte et al. (2014) compared the differences in the biology and morphology of two different Brazilian isolates of *A. cantonensis*, ac8 and ac9 haplotypes. The morphometric analysis showed that male and female specimens isolated from ac8 haplotype were significantly larger with respect to body length, oesophagus length, spicule length (male) and distance from the anus to the posterior body end (female) compared to specimens from ac9. Light microscopy of the copulatory bursa showed little variation in the split of lateral rays in the right bursal lobe between the two haplotypes. The biological, morphological and morphometric variations observed between the ac8 and ac9 haplotypes is in agreement with the variation at the molecular level using the cytochrome oxidase subunit I, suggesting different entries of these haplotypes in South America (Monte et al. 2014, 1061).

**Table 1. Morphometric data of *Angiostrongylus cantonensis* adult worms from natural or experimental infection of *Rattus norvegicus* and *Rattus rattus* from different states of Brazil (Rio de Janeiro, Santa Catarina, Pará and Pernambuco) (Maldonado et al. 2010; Moreira et al. 2013; Monte et al. 2014)**

State	Rio de Janeiro						Santa Catarina		Pará		Pernambuco	
Municipalities	São Gonçalo		Barra do Piraí		Cajú		Joinville		Belém		Escada	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Body length	19.40 ± 1.99	25.49 ± 0.59	18.48 ± 0.45	21.63 ± 0.56	20.27 ± 1.36	26.34 ± 1.13	15.39 ± 0.88	18.83 ± 0.54	21.9 ± 0.7	26.9 ± 1.7	24.65 ± 1.01	34.25 ± 2.09
Body width	0.32 ± 0.01	0.45 ± 0.01	0.26 ± 0.01	0.29 ± 0.01	0.32 ± 0.04	0.42 ± 0.06	0.26 ± 0.2	0.30 ± 0.02	0.33 ± 0.02	0.41 ± 0.02	0.34 ± 0.05	0.41 ± 0.05
Esophagus	0.24 ± 0.03	0.28 ± 0.01	0.27 ± 0.01	0.29 ± 0.01	0.25 ± 0.02	0.28 ± 0.02	0.29 ± 0.05	0.30 ± 0.03	0.31 ± 0.08	0.37 ± 0.02	0.28 ± 0.03	0.3 ± 0.02
Nerve ring	0.08 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.14 ± 0.03	0.15 ± 0.03	0.16 ± 0.09	0.15 ± 0.08	0.25 ± 0.01	0.28 ± 0.025	0.13 ± 0.04	0.14 ± 0.04
Excretory pore	0.31 ± 0.06	0.31 ± 0.06	0.37 ± 0.01	0.41 ± 0.01	0.35 ± 0.07	0.32 ± 0.06	0.16 ± 0.22	0.48 ± 0.15	0.43 ± 0.03	0.46 ± 0.02	0.3 ± 0.09	0.32 ± 0.05
Spicules	1.23 ± 0.06	-	1.25 ± 0.01	-	1.23 ± 0.09	-	1.33 ± 0.01	-	1.21 ± 0.17	-	1.29 ± 0.08	-
Gubernaculum	0.07 x 0.02	-	0.10 x 0.01	-	0.06 x 0.02	-	0.21 x 0.03	-	0.11 ± 0.008	-	0.05 x 0.01	-
Vulva-tail	-	0.15 ± 0.01	-	0.16 ± 0.01	-	0.19 ± 0.02	-	0.22 ± 0.06	-	0.20 ± 0.04	-	0.20 ± 0.05
Anus-tail	-	0.06 ± 0.01	-	0.06 ± 0.004	-	0.06 ± 0.02	-	0.06 ± 0.02	-	-	-	0.08 ± 0.03
Eggs	-	0.08 x 0.04	-	0.05 ± 0.03	-	-	-	0.1 x 0.06	-	-	-	-

## GENETIC VARIABILITY

Molecular biology techniques have been employed to detect *A. cantonensis* over the years. Different genetic markers have been used for this purpose, most initially targeted to ribosomal RNA genes (rDNA). However, there is limited genetic variation in the rDNA within the family Angiostrongylidae (Chan et al. 2015, 10-14; Barratt et al. 2016, 1101-1105).

Techniques to distinguish closely related *Angiostrongylus* spp. have been employed, such as PCR-restriction fragment length polymorphism (PCR-RFLP), targeting the mitochondrial cytochrome c oxidase subunit I (COI) and the internal transcribed spacer 2 (ITS2) DNA of Brazilian specimens (Caldeira et al. 2003, 1040; Caldeira et al. 2007, 888). PCR-RFLP targeting the ITS2 gene was also used to analyze the infection of *A. cantonensis* in *A. fulica* collected in peridomicile areas in Pernambuco (Thiengo et al. 2010), and to investigate the presence of this nematode in Brazilian port areas. In the latter study, the authors found snails naturally infected with *A. cantonensis* larvae at 11 of the 30 ports investigated (36.6%) (Carvalho et al. 2012, 742-745).

The conventional PCR assay has proved to be a useful tool to detect *A. cantonensis* in molluscs from Hawaii using the fragment of 18S rDNA, although cross-reactivity with other nematode species has been observed, since 18S rDNA is a highly conserved gene (Qvarnstrom et al. 2007, 1416-1417). Mitochondrial genes such as the cytochrome c oxidase subunit I (MT-COI) and NADH dehydrogenase subunit 6 genes represent possible targets for differentiating closely related *Angiostrongylus*, since they possess greater variability within populations (Eamsobhana et al. 2010b, 154; Gasser et al. 2012, 1886-1889; Lv et al. 2012, 122; 2014,175; Tokiwa et al. 2012,433-435; Yong et al. 2015, 68-69; Barratt et al. 2016, 1104). The 66-kDa protein gene of *A. cantonensis* was also a successful marker to distinguish three *Angiostrongylus* species, showing that *A. cantonensis* is a sister group to *Angiostrongylus malaysiensis*, although no clear intraspecific distinction was observed among *A. cantonensis* from Thailand, China, Japan and Hawaii (Eamsobhana 2010a, 568).

In a survey of genetic variation of *A. cantonensis* in Asia using small subunit (SSU) ribosomal RNA and (MT-COI) gene sequences, it was possible to determine two genotypes (G1 and G2) for SSU gene among 17 individuals from 17 different geographical localities. In contrast, eight haplotypes (ac1- ac8) were recorded for MT-COI gene among 83 specimens from 18 different localities (Tokiwa et al. 2012, 434-435). Based on these results, a similar study was carried out with *A. cantonensis* specimens collected in different geographical localities from Brazil using the MT-COI gene as a single genetic marker. The authors observed among the gene sequences the same haplotypes described by Tokiwa et al. (2012) and a new haplotype, named ac9, which referred to a specimen collected in the port area of Rio de Janeiro (Monte et al. 2012, 5).

More recently, the analysis using mitochondrial cytochrome b gene (CYTB) as genetic marker showed 11 haplotypes of *A. cantonensis* from eight locations in Thailand, suggesting multiple independent origins (Dusitsittipon et al. 2015, 549). The variation observed in that study was higher than that found in Japan, China and Taiwan (eight haplotypes) using MT-COI gene sequences (Tokiwa et al. 2012, 433-435). Additionally, Yong et al. (2015) analyzed the genetic relationships of geographical isolates of *A. cantonensis* from Thailand, China and Hawaii. They reported 18 haplotypes in the sequences from these three geographical locales, with Thailand represented by 15 haplotypes. The haplotypes from Thailand were distinct from those of *A. cantonensis* from China and Hawaii, agreeing with the findings based on COI nucleotide sequences (Tokiwa et al. 2012, 433-435). These studies highlight the importance of the CYTB gene as a promising marker for determining the genetic diversity of a given population/taxon in addition to the previously mentioned techniques.

## ECOLOGY

The dynamics of parasitic transmission in intermediate and definitive hosts are influenced by biotic and abiotic factors. A recent report of *A. cantonensis* in the Americas (Maldonado et al. 2012, 303) has generated

questions about the ecology of this parasite. Next is a short review of some studies focusing on the influence of ecological and host characteristics on infection by *A. cantonensis*.

## INTERMEDIATE HOSTS

*A. cantonensis* presents low specificity for its intermediate host (Maldonado and al. 2012, 309; Iwanowicz et al. 2015, 750) and various species of terrestrial and freshwater snails have been reported naturally infected with the rat lungworm around the world (Wallace and Rosen 1969, 210-212; Caldeira et al. 2007, 888; Thiengo et al. 2010). The giant African snail *A. fulica* is one of the most frequent intermediate hosts, but other molluscs and slugs have been found to be infected with *A. cantonensis*, such as *Bradybaena similaris*, *Subulina octona*, *Pomacea canaliculata* and *P. lineata* (Maldonado et al. 2012, 309).

Recently, a seasonal study correlating the parasitological parameters of prevalence and abundance of infection by *A. cantonensis* in *A. fulica* and *B. similaris* and climatic variables in Rio de Janeiro observed that *A. fulica* presented high prevalence of infection during two years, and that the prevalence and abundance increased mainly in the dry season (Oliveira et al. 2015, 739). Interestingly, at a specific site of the study, the prevalence of *A. cantonensis* and the abundance of *A. fulica* decreased together. These authors suggested that *A. fulica* plays a major role in parasite transmission and *B. similaris* acts as an auxiliary intermediate host to maintain the parasite infection in that locality. Furthermore, rainfall, temperature, seasonality and shell size of *A. fulica* did not present significant relationships with *A. cantonensis* abundance.

Epidemiologic studies in the United States reported molluscs naturally infected with *A. cantonensis* (Emerson et al. 2013, 792; Iwanowicz et al. 2015, 750). The authors postulated that climate change is a possible factor influencing the spread of *A. fulica* infected with *A. cantonensis* in Florida. York et al. (2014) formulated an ecological niche model for the distribution

of *A. cantonensis* and predicted that the habitats situated in tropical to subtropical regions are the most suitable.

Another ecological study involving molluscs naturally infected molluscs with *A. cantonensis* was reported by Ibrahim (2007) in Sinai, Egypt. The influence of salinity, season and host size on the prevalence, mean intensity and mean abundance of *A. cantonensis* in freshwater and terrestrial molluscs suggest salinity intolerance of *A. cantonensis* L<sub>1</sub> larvae. Moreover, during spring and summer seasons the prevalence and intensity of *A. cantonensis* infection was higher in terrestrial molluscs while the larger gastropod *Lanistes carinatus* was positively correlated with mean abundance and mean intensity of infection, suggesting that molluscs with higher body mass harbor more parasites.

## DEFINITIVE HOST

The genus *Rattus* is known to be the main definitive host of *A. cantonensis* (Wang et al. 2012, 392). The species most frequently reported as being involved in lungworm transmission are *Rattus norvegicus* and *R. rattus* (Maldonado et al. 2012, 310). They play a crucial role in the transmission of the parasite, but little is known about the environmental and host features that can influence the transmission of *A. cantonensis* in the definitive host in the New World.

The first long-term study focusing on the pattern of *A. cantonensis* infection in *R. norvegicus* in the Americas was performed in Rio de Janeiro, Brazil (Simões et al. 2014, 2). They investigated the influence of season, gender and host weight on the parasite prevalence and intensity of infection. Interestingly, the prevalence was high along the two years of study, confirming the stability of *A. cantonensis* transmission. In addition, female rodents presented higher rate of prevalence and abundance of infection. These findings suggest the importance of female behavior and season on the parasite transmission dynamics.

The prevalence of *A. cantonensis* and *A. mackerrasae* was also evaluated in Australia in introduced and native rats (*R. rattus*, *R. norvegicus*,

*R. fuscipes* and *R. lutreolus*). The findings indicated an increase of infection due to new and repeated infections and durability of the adult worm in rats. Furthermore, high prevalence was also observed in subsequent months in the rainy season, which might have been associated with the abundance of the intermediate hosts (Aghazadeh et al. 2015, 327-328).

However, it is difficult to predict which variables (biotic or abiotic) can influence the parasite transmission in the definitive or intermediate hosts, since there are different ecological factors in each studied area. Furthermore, the hosts' features respond differently in certain environmental conditions. Thus, experimental and field studies should be associated to better understand the influence of these variables on the *A. cantonensis* transmission dynamics.

## **PARASITE-INTERMEDIATE HOST RELATIONSHIP**

Two molluscs models of molluscs have been used to study the relationship between *A. cantonensis* and its intermediate host, *Biomphalaria glabrata* and *A. fulica* (the last found naturally parasitized by *A. cantonensis*). These two models present the greatest amount of information on metabolic and physiological changes during *A. cantonensis* infection (Tunholi-Alves et al. 2015, 1).

*B. glabrata* experimentally infected by 1,000 larvae ( $L_1$ ) of *A. cantonensis* showed significant decrease in the concentrations of triacylglycerol (TAG) and cholesterol and increase in the neutral lipids in the digestive gonad-gland (DGG) (Tunholi-Alves et al. 2013, 2113). Moreover, the infected groups presented a decrease in the hemolymph content of glucose, calcium, total proteins and uric acid and an increase in the urea content and enzymatic activity of lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) compared to the control group (Tunholi-Alves et al. 2013, 2114; Tunholi-Alves et al. 2015, 3). Differently, *A. fulica* experimentally infected by different loads of *A. cantonensis* demonstrated an increase in the

concentration of urea, uric acid and total protein and a decrease of ALT and AST activity (Tunholi-Alves et al. 2013, 2114; 2015, 3).

Variations in the polysaccharide reserves were also demonstrated in molluscs, with infected groups showing a decrease in glycogen reserves of the cephalopedal mass and digestive gland and of galactogen in the albumin gland, indicating impairment of the snail's nutritional status. Together, the results confirm the involvement of homeostatic biochemical mechanisms, characterized mainly by the activation of protein metabolism, in infected molluscs (Tunholi-Alves et al. 2011, 222).

Alterations in the levels of protein metabolism can partly explain the higher concentrations of urea, uric acid and calcium in the hemolymph of infected molluscs. In addition, the increased activity of the enzymes LDH and transaminases (ALT and AST) in infected organisms suggests the involvement of a fermentative pathway in energy production, and an important association between protein metabolism and the formation of glucose through gluconeogenesis. Furthermore, due to nutrient depletion by the host and the ability of larvae to utilize the host's lipids as energy substrate, marked changes in the reproductive biology of infected *B. glabrata* were observed. The decrease of reproductive capacity of infected molluscs has been associated with starvation state observed during infection (Becker 1980, 104-106; Tunholi-Alves et al. 2011, 222; Tunholi-Alves et al. 2013, 2115; 2014a, 67; Tunholi-Alves et al. 2015, 4).

The infection with *A. cantonensis* caused intense cellular disorganization in the cephalopedal mass and digestive gland, characterized by granulomatous reactions and hemocyte infiltration. These tissue changes may be related to the difficulty of molluscs to normalize their energy metabolism, due to the inability of damaged tissue to synthesize and replenish glycogen stores (Harris and Cheng 1975, 521-528; Tunholi-Alves et al. 2013, 2115; 2014b, 164).

## **PARASITE-DEFINITIVE HOST RELATIONSHIP**

Wistar rats (*R. norvegicus*) experimentally infected with 100 L<sub>3</sub> of *A. cantonensis* demonstrated hepatic and histopathological alterations, characterized by increased metabolic activity of ALT, AST, GGT and ALKP enzymes. Changes in glyceic metabolism, with higher levels of hepatic glycogen lower levels of serum glucose, were also observed. The liver of infected rats showed ductal and portal hyperplasia associated with cellular infiltration with mature eosinophils and plasmocytes, including 31–47% increase in the liver weight (Garcia et al. 2014b, 40-41).

The aminotransferases ALT and AST are normally present in low concentrations in the serum (Kaplan 1987, 219–260). The increased activity of these enzymes is suggestive of hepatopathies (Andrioloand Borges, 1989). The increase of these aminotransferase levels can be due to the high sensitivity to a wide range of injuries and induction of hepatic or extra-hepatic changes. Furthermore, this increase can also result from the release of worms' excretion-secretion products, leading to increased permeability of the hepatocyte membrane, allowing these enzymes to enter the bloodstream (Rej, 1978; Kaplan 1987, 219–260; Burtis et al. 2006).

Activity of glycolytic enzymes in juvenile specimens of *A. cantonensis* was observed by Shih and Chen (1982), indicating the presence of an oxidative pathway to maintain the parasite's energy balance. This aspect highlights the importance of glucose monomers for the production of energy required for the metabolic processes of helminths.

The hematological parameters of rats infected with *A. cantonensis* demonstrated significant alterations: hematocrit, red blood cell (RBC) and platelet counts were significantly lower. Significant differences between infected and uninfected groups occurred only in hemoglobin levels and mean corpuscular hemoglobin (MCHC). The white blood cell analysis revealed a significant increase in the number of neutrophils, eosinophils, basophils, lymphocytes and total leukocytes (Garcia et al. 2014a, 633-634).

The levels of cardiac enzymes such as creatine kinase (CK), creatine kinase MB fraction (CK-MB) and lactate dehydrogenase (LDH) increased significantly. Hemogasometric analysis detected decline of pH, pCO<sub>2</sub>, pO<sub>2</sub>

and sO<sub>2</sub> in the infected rodents (Garcia et al. 2014a, 633-634; 2014b, 40-41).

These results demonstrate that infection caused by *A. cantonensis* in *R. norvegicus* promotes significant haematological changes, manifested mainly in the form of regenerative anemia, thrombocytopenia and eosinophilia. Additionally, histopathological changes in the pulmonary parenchyma from infected rodents and the occurrence of areas of necrosis and extensive fibrosis may be directly related to the development of cellular hypoxia (Chikweto et al. 2012, 161; Vannucchi et al. 2012, 109; Garcia et al. 2014a, 634; 2014b, 41).

## CONCLUSION

In conclusion, in recent years there has been an increase in knowledge about the parasite *A. cantonensis*, which includes its relationship with the intermediate and definitive hosts and the role of the environment in the regulation of transmission dynamics. However, there are still gaps in knowledge that await elucidation, such as variation in the morphology, genetic variability, infection pattern under distinct environmental conditions, and metabolic and physiological alterations in intermediate and definitive hosts. Therefore, further research is necessary to shed light on the infection not only of humans but also domestic and wild animals.

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