International Journal for Parasitology: Parasites and Wildlife 4 (2015) 323–328

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

A survey of *Angiostrongylus* species in definitive hosts in Queensland

Mahdis Aghazadeh [a, b, *], Simon A. Reid [c], Kieran V. Aland [d], Angela Cadavid Restrepo [e], Rebecca J. Traub [f], James S. McCarthy [b, c], Malcolm K. Jones [a, b]

[a] School of Veterinary Science, University of Queensland, Gatton, QLD 4343, Australia
[b] QIMR Berghofer Medical Research Institute, Brisbane, QLD 4006, Australia
[c] School of Public Health, University of Queensland, Herston, QLD 4006, Australia
[d] Queensland Museum and Sciencentre, QLD 4101, Australia
[e] School of Population Health, Australian National University, ACT 0200, Australia
[f] Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, VIC 3052, Australia

ARTICLE INFO

Article history:
Received 17 May 2015
Received in revised form
24 June 2015
Accepted 26 June 2015

Keywords:
Angiostrongylus cantonensis
Angiostrongylus mackerrasae
Rat lungworm
Australia
Rattus rattus
Southeast Queensland

ABSTRACT

Despite the recent sporadic reports of angiostrongyliasis in humans, dogs and wildlife in eastern Australia there has been no systematic study to explore the epidemiology of *Angiostrongylus* spp. in definitive and intermediate hosts in the region. Little is known about the epidemiology of *Angiostrongylus* species in the definitive host in southeast Queensland, since the only survey conducted in this region was performed in the late 1960s. In this study, free-living populations of *Rattus* spp. were sampled and examined for the presence of adult and larval *Angiostrongylus* in the lungs, and of larvae in faeces. The prevalence of infection with *Angiostrongylus* spp. was 16.5% in *Rattus* spp. trapped in urban Brisbane and surrounds. This prevalence is much higher than estimates of earlier studies. This highlights the possible risk of zoonotic infection in children, dogs and wildlife in this region and indicates the necessity for public awareness as well as more detailed epidemiological studies on this parasite in eastern Australia.

1. Introduction

Angiostrongylus is a genus of nematode belonging to the superfamily Metastrongyloidea. The genus contains species characterised by a two-host life cycle that always involves a terrestrial and aquatic mollusc as intermediate host (Carreno and Nadler, 2003). All species of *Angiostrongylus* live in the arteries of their definitive host and some have tropism to the central nervous system (CNS) in at least at one stage of their lifecycle in the mammal host. The well-studied lungworm, *Angiostrongylus cantonensis* causes severe and sometimes fatal neurologic disease in accidental hosts, including humans, domestic animals and wildlife. In humans, the clinical features involving CNS, include severe headache, radiculomyopathy and paralysis of cranial nerves (Cooke-Yarborough et al., 1999), while in dogs, clinical signs include hyperesthesia, hind limb paresis and death (Mason et al., 1976). Definitive hosts are infected by the ingestion of infected molluscs containing third-stage larvae of the parasite (Bhaibulaya, 1991) or the mucus secreted from infected mollusc (Heyneman and Lim, 1967). For accidental hosts, it was thought that ingested *Angiostrongylus* larvae migrate only as far as the brain. However, a recent study showed that larvae may also continue to migrate to the pulmonary circulation and complete their development into adults in human (Lindo et al., 2004; Cui et al., 2011).

Another species of rat lungworm, *Angiostrongylus mackerrasae* also occurs in Australia. Although there is no direct evidence that *A. mackerrasae* infects humans, it has recently been reported as a cause of severe lung pathology in a native flying fox (Pteropus alecto) (Mackie et al., 2013). So far definitive speciation of parasites that have caused human disease has been attributed to *A. cantonensis*; however, this assumption needs to be confirmed due to occurrence of *A. mackerrasae* in Australia.

Although terrestrial molluscs are not commonly eaten as food in Australia, isolated incidences of individual cases of human neuro-angiostrongyliasis have occurred due to the accidental or voluntary (as a bet) ingestion of an infected mollusc (Senanayake et al., 2003; Blair et al., 2013; Morton et al., 2013). In particular, *A. cantonensis* has been recognised as a cause of significant disease, especially in children (Li et al., 2001). In the last few years, human cases have been reported from New South Wales (Senanayake et al.,

* Corresponding author. QIMR Berghofer Medical Research Institute, Brisbane, QLD 4006, Australia.

http://dx.doi.org/10.1016/j.ijppaw.2015.06.003

2003; Spratt, 2005; Blair et al., 2013; Morton et al., 2013), raising concerns that neuro-angiostrongyliasis may be emerging as a more significant public health issue in eastern Australia.

Current knowledge of the epidemiology *Angiostrongylus* spp. throughout Australia is limited to small surveys of rats conducted in Queensland in the 1950s and 1960s (Mackerras and Sandars, 1955; Bhaibulaya, 1968), a survey of rodents in the Gulf of Carpentaria in late 60s (Dunsmore, 1968), two surveys of dogs in Queensland and New South Wales (Mason, 1987; Lunn et al., 2012) and a survey of rats in Jervis Bay in New South Wales between 2003 and 2005 (Stokes et al., 2007). Most of the above rodent surveys were performed in non-urban areas, distant from human habitation and, therefore, may not reflect the potential risk of human infection.

The prevalence, distribution and the potential hotspots for transmission of *Angiostrongylus* spp. are unknown in eastern Australia. Hence this study aimed to place a step forward to investigate the presence of *Angiostrongylus* infection in the definitive host among randomly selected populations of *Rattus* spp. in southeast Queensland where the parasite has been previously reported.

2. Materials and methods

2.1. Sample collection

This study was approved by the QIMR Berghofer animal ethics committee (Project Ethic No: A1208-607M). Introduced rats (*Rattus rattus* and *Rattus norvegicus*) and native rats (*Rattus fuscipes* and *Rattus lutreolus*) were trapped in different areas in and around Brisbane (mostly urban areas) between June 2012 and January 2015 (Fig. 1) (under permit from the Department of Environment and Heritage Protection of the Queensland Government: WIS12109412. Brisbane is a city located in southeast Queensland, Australia where the average annual temperature ranges between 16.2 °C and 26.4 °C and the average annual rainfall is approximately 1149.1 mm. An additional survey was conducted in Cairns, north Queensland in May 2014 where the annual temperature ranges between 20.8 °C and 29.0 °C and the average annual rainfall is approximately 2010.7 mm. Trap sites were chosen non-randomly based on the reported presence of *Rattus* species.

Wire cage traps baited with peanut butter and rolled oats were placed along walls or fences at least 2 m apart for at least one week with nightly rebaiting. The location of the traps was recorded using spatial coordinates obtained by global positioning system (GPS). Each trapped rat was euthanized on site using a portable carbon dioxide chamber. Rats were then transported to the laboratory where the lungs and pulmonary vasculature were examined for the presence of adult lungworms. Each adult male *Angiostrongylus* recovered from lungs of positive rats was examined to determine the species based on the spicule length of male worm described by Bhaibulaya (1968) as illustrated by Aghazadeh et al. (2015) and here (Fig. 2). All the recovered *Angiostrongylus* spp were preserved in 70% ethanol for later molecular testing. Samples of kidney, liver, spleen and faeces were also collected from each rat and stored at −20 °C for further analyses. The dimensions of the spleen were recorded for each rat and splenic size was calculated using the formula described by Araujo et al. (2014) in order to investigate any association between *Angiostrongylus* infection and splenomegaly.

The prevalence of *Angiostrongylus* spp. in intermediate hosts was determined by sampling mollusc species from different areas in Brisbane and surrounds, and digested using an artificial gastric solution (2 g of 1:3000 Pepsin: Amresco LLC, OH, USA and 8 ml of 36% HCl in 1 L of water). The digests were then visually examined using light microscopy to identify first to third-stage larvae of *Angiostrongylus* spp.

2.2. Data analysis

The associations between *Angiostrongylus* infection, the worm burdens and rat body weight, maturity for both rodent groups (introduced or native) were evaluated using the Chi-square test (for dichotomous variables) and Independent T-test (continuous

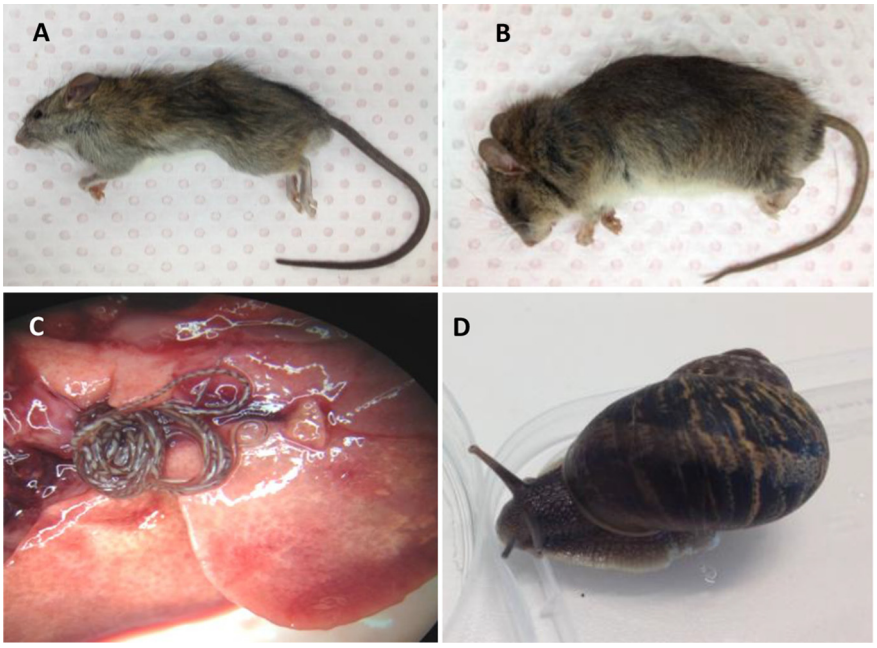

Fig. 1. Images of two principal *Rattus* spp., *Angiostrongylus cantonensis* and its putative intermediate host from a survey conducted in Brisbane, Australia, 2012–2014. A: *Rattus rattus* was the most prevalent species of rat found in this survey. B: *Rattus fuscipes*. C: Adult *A. cantonensis* in pulmonary arteries of *Rattus rattus*. D: *Helix aspersa* found is Brisbane harbouring *Angiostrongylus* larvae.