International Journal for Parasitology: Parasites and Wildlife 5 (2016) 258–262

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

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

The life cycle of the reptile-inhabiting nematode *Abbreviata hastaspicula* (Spirurida: Physalopteridae: Physalopterinae) in Australia

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ARTICLE INFO

Article history:
Received 14 April 2016
Received in revised form
23 August 2016
Accepted 25 August 2016

Keywords:
Lifecycle
Abbreviata spp.
Abbreviata hastaspicula
Nematodes
Lizards
Physaloptera
Insects
Host-parasites

ABSTRACT

This study elucidates the life-cycle of the reptile inhabiting nematode *Abbreviata hastaspicula* (Spirurida: Physalopteridae: Physalopterinae) in Australia. Eight *Varanus gouldii* (Lacertilia: Varanidae), and two *Christinus marmoratus* (Reptilia: Gekkonidae) lizards were captured in the wild. Two *V. gouldii* were used as controls and no experimental procedures were carried out on them. Another six *V. gouldii* (final host) and the two *C. marmoratus* (paratenic host) were treated with oral anthelmintics to remove all parasitic worms and were fed with infected live arthropods containing third stage larvae of *Abbreviata hastaspicula.* Faeces of *V. gouldii* were examined under the microscope weekly to determine whether the third stage larvae had developed into adults. Two months later, a total of 30 larvae and adults of *A. hastaspicula* were found in the stomachs of four experimentally-infected *V. gouldii* lizards. No cysts or larva were found in the *C. marmoratus.* This is the first study to demonstrate the life-cycle of this genus of nematode in their definitive reptile hosts.

1. Introduction

The nematode *Abbreviata hastaspicula* (Spirurida: Physalopteridae) occurs predominantly in *Varanus gouldii* lizards, principally in the arid interior of Australia. It requires an arthropod intermediate host to complete its life-cycle (Mönnig, 1934, Alicata, 1937).

Distribution of the nematode genus *Abbreviata* is worldwide (Bain et al., 2015), in Australia, nematodes in this genus are widespread. They are most common in the lizard fauna (Jones, 2014), and physalopterid nematodes also occur in birds (Berger, 2010). The arid Australian landscapes support the richest and the most diverse lizard fauna in the world, due to the dry hot climate and the dominant, hummock grasslands (*Triodia* spp.), which provide various habitat niches for several species of lizard (Pianka, 1986). In addition, a range of shrubs and sparse trees provide niches for a great variety of both diurnal and nocturnal lizards (Rich and Talent, 2008). The *Varanidae* contain the world's largest lizards, with at least 25 endemic described species in Australia (Bush et al., 2000). The species of lizard in this study, *Varanus gouldii* is found in all areas of Western Australia except the coolest and wettest parts.

Factors affecting the geographical pattern of *Abbreviata* are probably firstly, the distribution of suitable arthropod intermediate hosts; secondly, the ability of the eggs to survive and remain viable outside the final hosts (Jones, 2014); thirdly, the availability of prey for the hosts e.g. small lizards. A fuller understanding of the biology of species of Physalopterinae would clarify the relative importance of these factors.

The life-cycle of spirurid nematodes consists of six stages involving an egg, four larval stages (or juvenile stages) and lastly, adult. For parasitic nematodes found in vertebrates, the infective stage is always the third larval stage, L3 (Anderson, 2006). Similar to other spirurid nematodes, species of *Abbreviata* exhibits a heteroxeous life cycle. However, in paratenic hosts, the infective stage of a parasite persists without essential development and usually lack of growth (Anderson, 2006; Anderson et al., 2009). Its lifecycle can only be completed by the final host (larger lizards) feeding on insects (intermediate hosts) or paratenic hosts (smaller lizards) containing infective third stage larvae (Anderson, 2006; Preston and Johnson, 2010). Larvae ingested by possible paratenic hosts generally encyst in the abdominal tissues, where they can persist until they are eaten by a predaceous final host. The ingested larvae then attach to the stomach wall and, depending on the amount of food in the stomach, grow to adult (Lee, 1955). *Abbreviata* requires the tropical cricket, *Teleogryllus oceanicus* to act as its intermediate host is the only partial life cycle of *Abbreviata* spp. that is known from Australia (King et al., 2013). This study was undertaken to

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http://dx.doi.org/10.1016/j.ijppaw.2016.08.002

elucidate the life-cycle of *Abbreviata hastaspicula* in Australia by infecting wild caught*Varanus gouldii* (final host) and *Christinus marmoratus* (paratenic host) with live arthropods that had been infected with the larvae of *Abbreviata hastaspicula*.

2. Materials and methods

2.1. Infecting the lizards

Eight *Varanus gouldii* were caught from arid Paynes Find (latitude:−29° 43′ 21.5436″, longitude: 117° 10′ 24.3912″) and the two *Christinus marmoratus* were caught at Wooroloo (latitude:−31° 48′ 7.3218″, longitude: 116° 18′ 51.6384″), Western Australia with the permission of Department of Environment and Conservation Australia (Licence no. SF009524). The average weight of the varanids was 4.15 ± 0.52 kg and that of the geckos was 17.1 ± 3.20 g. The *Varnaus* were marked from no. 1 to 8.

The faeces of the wild-caught *V. gouldii* were collected from the floor of their individual housing cage and were checked weekly for eggs of the nematode *Abbreviata hastapicula* Two *V. gouldii* (no. 1 and 2) were used as controls and were euthanized by sodium pentobarbital injection (dose: ≥ 100 mg/kg) as soon as embryonated eggs were found in the faeces to ascertain that nematodes in the experimental lizards were the same as the species of nematodes that occurred in these lizards in the wild.

The remaining lizards (six *V. gouldii* and two *C. marmoratus)* were treated with Panacur 25 (0.4 ml/100 g body weight orally once and repeat in 14 days). One week after the two doses of Panacur 25 were administered to all the lizards directly with an oral syringe, no eggs were found in the *V. gouldii* (no. 3–8) , thus ascertaining that all six *V. gouldii* and the two geckoes were free of parasites.

After ascertaining that all the six *V. gouldii* were free from nematodes, two *Varanus* (no. 3 and 4) were kept uninfected and parasite free, they were fed the two *C. marmoratus* three months later in this experiment. Four uninfected *Varanus* (no. 5, 6, 7 and 8) and two *C. marmoratus* were fed with infected live *Teleogryllus oceanicus* (Orthoptera: Ensifera: Gryllidae) crickets that ingested the embryonated eggs of *A. hastaspicula* 28 days earlier following the methodology previously described (King et al., 2013) (feeding of arthropods was a one-off event). Each *Varanus* consumed 19 *T. oceanicus* crickets that had contained third stage larvae of *Abbreviata hastaspicula,*and five *T. oceanicus* were given to each *C. marmoratus*. The *V. gouldii* had ingested all the *T. oceanicus* crickets as soon as they were given to them. The two *C. marmoratus* had broken apart and ingested some shattered parts of the crickets, but it was not certain whether they had ingested the infected cysts.

Faeces of *Varanus* were examined microscopically every week to determine whether the third stage larvae had developed into adults.

Two months later, in order to confirm that *Abbreviata hastaspicula* larvae can only develop into adults in a final host, the four infected *V. gouldii* (no. 5, 6, 7 and 8) were euthanized by sodium pentobarbital injection ,when the eggs of *A. hastaspicula* were found in their faeces.The two *C. marmoratus* (possible paratenic host) were euthanized by carbon dioxide inhalation and thier stomachs and gastrointestinal tracts were opened to check whether they contained larval cysts of *A. hastaspicula*.

After another month, the two *V. gouldii* (no. 3 and 4) that had consumed the two *C. marmoratus* were euthanized by injection of sodium pentobarbital and were dissected (UWA Animal Ethics Ref. RA/3/100/1248).

2.2. Dissecting the lizards

The lizard was laid on its back, and a vertical ventral incision was made from the sternum to the pubis. The connective tissue was peeled from skin and turned back so that viscera were exposed. The lower oesophagus, stomach and intestine were released from connective tissues and examined for adult nematodes and larvae. The stomach was opened by vertical incision, food was noted and collected. Stomach, gastrointestinal tracts, cysts and worms were removed with forceps and fixed in formalin and preserved in 70% alcohol.

Adult nematodes of *A. hastaspicula* found in *V. gouldii* (no. 5, 6, 7 and 8) were observed under light microscopy under X4, X10 and X20 objectives after clearing in chlorolactophenol, and lengths of the larvae and adult nematodes (in mm) were measured. Their stage of development was assessed by the differentiation of their sexual organs (Cawthorn and Anderson, 1977).

2.3. Histology staining

Nematodes and small segments of stomach and gastrointestinal tracts of infected *Varanus* were dehydrated, embedded in paraffin, and serially sectioned at 5 μm. Tissue samples from animals were stained with hematoxylin and eosin (Gabe and Blackith, 1976).

3. Results

3.1. Infection prevalence and intensity rate of A. hastaspicula in V. gouldii and C. marmoratus before the experiment

Embryonated eggs of *A. hastaspicula* were present in the faeces of all wild-caught *V. gouldii*, indicating that all the *V. gouldii* were infected with this nematode in the wild. Embryonated eggs of *A. hastaspicula* and *A. antarctica* were found in one of the controls *V. gouldii*. The density of eggs in the faeces was 4 eggs/2 mg of faeces. For the *C. marmoratus*, because this species of Gekkonidae could be a possible paratenic host that might contain infective third stage larvae but not adult *A. hastaspicula*, and eggs could only be produced by adult *A. hasataspicula,* we therefore were not able to tell whether the *C. marmoratus* have infection as we could not observe this in the faeces.

3.2. Infecting the experimental lizards

Two months after the lizards had ingested infected *T. oceanicus*, eggs of *A. hastaspicula* were being passed in the faeces of the experimentally infected *V. gouldii* (no. 5, 6, 7 and 8). The infection rate for the final host was 100%. The numbers of *A. hastaspicula* found in the experimental *V. gouldii* no. 5, 6, 7 and 8 were 5, 3, 8 and 14 respectively. No larva or adult nematodes of *A. hastapicula* were found in *C. marmoratus*. Concurrent infection with *A. antarctica*

Fig. 1. A large number of *Abbreviata hastapicula* were found in the stomach of a control *Varanus gouldii.*