Experimental studies on the life-cycle of *Sebekia wedli* (Pentastomida: Sebekidae)

K. JUNKER[1], J. BOOMKER[2]* and D.G. BOOYSE[2]

ABSTRACT

JUNKER, K., BOOMKER, J. & BOOYSE, D.G. 1998. Experimental studies on the life-cycle of *Sebekia wedli* (Pentastomida: Sebekidae). *Onderstepoort Journal of Veterinary Research*, 65:233–237

Four young Nile crocodiles (*Crocodylus niloticus*) were infected with infective pentastome larvae obtained from naturally infected Mozambique bream, *Oreochromis mossambicus*, and red-breasted bream, *Tilapia rendalli swierstrai* in the Kruger National Park. At day 95 post infection one of the crocodiles died and three female and four male *S. wedli* were recovered from its lungs. One pair was found *in copula* but the uteri of the females were not yet developed. Males and females were of about equal size. After 226 d post infection the three remaining crocodiles were sacrificed. Two of these harboured no pentastomes but eight were taken from the lungs of the third. The sex ratio had shifted in favour of the females, seven females and one male being present.

One of the females recovered from the crocodiles was placed in saline and produced 3 400 eggs. These were used to infect eight guppies, *Poecilia reticulata*. Within 31 d two infective stages of *S. wedli* had developed in one of the guppies thus completing the life-cycle of the pentastome. *S. wedli* recovered from experimentally infected final hosts were slightly smaller than those recovered from a wild-caught final host.

Keywords: *Caiman sclerops*, *Crocodylus niloticus*, life-cycle, pentastomes, *Sebekia oxycephala*, *Sebekia wedli*, *Subtriquetra subtriquetra*

INTRODUCTION

Although it has been known for a long time that pentastomes infecting crocodiles use fish as intermediate hosts, little information is available on the development of these parasites in either the intermediate or the final host. We are aware of only two papers dealing with the life-cycle of crocodile pentastomes.

Winch & Riley (1986a) investigated the larval development of *Sebekia oxycephala*, by experimentally infecting fish with eggs derived from wild-caught South American caiman, *Caiman sclerops*. Winch & Riley (1986b) succeeded in obtaining primary larvae of *Subtriquetra subtriquetra* from the nasopharynx of *C. sclerops* and studied the subsequent developmental stages, up to the infective larva. The genus *Subtriquetra* is unique in that its primary larva is free-living and needs to make contact with the intermediate host (Vargas 1975; Winch & Riley 1986b), while the remaining pentastomids pass eggs into the water with the faeces or the sputum of the final host. The eggs must be ingested by an intermediate host for the primary larvae to hatch (Riley 1986).

This paper describes the results of experimental infections of fishes and four young Nile crocodiles, *Crocodylus niloticus*, with *Sebekia wedli* and forms part of a study conducted in the Kruger National Park in South Africa (Junker 1996).

* Author to whom correspondence is to be directed

[1] Department of Veterinary Pathology, Medical University of Southern Africa, Medunsa, 0204 South Africa

Present address: Vorwerkstr. 18, 76661 Philippsburg, Germany

[2] Department of Veterinary Pathology, Medical University of Southern Africa, P.O. Box 59, Medunsa, 0204 South Africa

Accepted for publication 20 August 1998—Editor

MATERIAL AND METHODS

Mozambique bream, *Oreochromis mossambicus*, and red-breasted bream, *Tilapia rendalli swierstrai*, were caught with baited hand lines in the Phabeni Dam (25°1′S, 31°15′E) in the Kruger National Park during February 1995 (Junker, Boomker & Booyse 1998). The fish were opened by ventral incision, and the viscera removed and examined under a stereoscopic microscope for the presence of pentastome larvae. The latter were removed, placed into normal saline and kept in a refrigerator at 4–8 °C.

Four young Nile crocodiles, 70–95 cm long, were obtained from a breeder in the North West Province. From February to April 1995 (summer/autumn), the crocodiles were kept in an outdoor enclosure, measuring 3 x 2,5 m. Two-thirds of this enclosure was filled with water to a depth of 30 cm and the remainder was filled with 80 cm of river sand, sloping towards a shelter against the sun. From May to August (winter/spring) the crocodiles were transferred to another outdoor enclosure measuring 3,6 x 1,8 m. This enclosure had a sand and lawn bottom, and was equipped with a sunken, heated asbestos pool, measuring 1,6 x 0,75 x 0,3 m. Water was exchanged regularly to prevent the build-up of detritus. Water temperatures in the pool during winter ranged from 15 °C at night to 20 °C during the day. Air temperatures ranged from >30 °C in summer to freezing levels at night in winter. The crocodiles were fed three times a week with diced chicken, including the bones, or freshly killed laboratory-bred white mice.

Experimental infection of the final hosts

The crocodiles were infected according to the programme below, by gently force-feeding with a piece of fresh chicken meat into which the infective larvae were placed.

Crocodile 1: Twenty encysted infective larvae of *S. wedli* from *T. rendalli*, collected 3 d prior to infection

Crocodile 2: Seven encysted larvae of *S. wedli* from *O. mossambicus*, collected 5 d prior to infection

Crocodile 3: Six free-living larvae of *Subtriquetra* from *O. mossambicus*, collected 3 and 4 d prior to infection

Crocodile 4: Twenty encysted infective larvae of *S. wedli T. rendalli*, collected 5 d prior to infection

Crocodile 1 was found dead 95 d post infection (p.i.) and examined approximately 12 h after death. Crocodiles 2, 3 and 4 were sacrificed at 226 d p.i. and dissected immediately after death. The lungs, tracheas and hearts of the crocodiles were placed into separate receptacles containing normal saline and examined under a stereoscopic microscope. Pentastomes were removed from the tissues by blunt dissection and all but one female from crocodile 4 were fixed in 70% ethanol.

For identification, the specimens were mounted in Hoyer's medium and all measurements were taken from whole-mounted specimens according to the methods described by Riley, Spratt & Winch (1990).

Experimental infection of the intermediate hosts

The female *S. wedli* recovered from the lungs of crocodile 4 was placed into saline for an hour and it produced 3400 eggs. The eggs, 70% of which contained fully developed primary larvae, were concentrated to a final concentration of 68 eggs/mℓ water.

Guppies, *Poecilia reticulata*, were used as experimental intermediate hosts. Two groups of four guppies each were infected over a 24 h period by placing each group into a beaker filled with approximately 400 mℓ water to which 1 mℓ of the egg suspension was added. Air stones were placed in the beakers to provide oxygen for the fish and to keep the eggs in suspension. After 24 h the groups of fish were transferred to separate aquaria, kept at a constant temperature of 26 °C and fed a commercial fish food twice daily.

RESULTS

Development in the final host

No pentastomes were recovered from crocodiles 2 and 3.

Seven of the 20 infective larvae given to crocodile 1 were recovered from its lungs. The infective larvae had developed into three females and four sub-adult males. One pair was found *in copula*, but separated *ex situ* revealing the long, thin cirrus threads of the male. Eggs were not present in the uteri of any of the females. At this stage, males and females were of approximately equal size (Table 1).

Seven females and one male *S. wedli* were recovered from crocodile 4, which gives a 40 % recovery rate. Whereas the sex ratio in crocodile 1 was balanced, it had shifted in favour of the females in crocodile 4. The uteri of the females were filled with eggs, containing fully developed primary larvae (Table 1).

Adult *S. wedli* from the experimentally infected hosts were compared with those from a naturally infected crocodile and found to be slightly smaller in some respects (Table 1).

Development in the intermediate host

Seven out of the eight guppies failed to become infected. However, one guppy became moribund 31 d

TABLE 1 A comparison of the main characteristics of *Sebekia wedli* recovered from experimentally infected crocodiles and crocodiles wild-caught in the Kruger National Park. All measurements are in micrometres unless otherwise indicated

Host	Parasite									
	Days post infection	Sex and number of specimens examined	Body length (mm)	Mouth dimensions			Hook length	Fulcrum length	Copulatory spicules	
				Overall length	Cadre length	Cadre width			Total length	Cowry shell length
Crocodile 1 (experimental)	95	F ($n = 2$)	7; 9	282; 283	194; 196	85; 87	76; 78	177; 162	NA	NA
	95	M ($n = 2$)	6; 7	206; 213	137; 132	69; 67	58; 66	133; 146	284; 309	190; 205
Crocodile 4 (experimental)	226	F ($n = 7$)	12 ± 2,7	285 ± 25,9	191 ± 18,5	105 ± 13,1	78 ± 2,5	167 ± 12,9	NA	NA
	226	M ($n = 1$)	6	211	140	62	60	NT	274	216
Wild-caught crocodile	?	F ($n = 14$)	11 ± 3,6	320 ± 51,3	215 ± 32,5	114 ± 18,5	79 ± 6,8	175 ± 17,0	NA	NA
	?	M ($n = 8$)	7 ± 1,4	221 ± 19,2	148 ± 8,1	79 ± 4,5	61 ± 3,9	139 ± 23,8	315 ± 32,5	214 ± 16,2

F = Female
M = Male
NA = Not applicable
NT = Not taken
? = Not determinable

p.i. and during its subsequent dissection two larvae of *S. wedli* were recovered. They were not yet encysted, but moved freely in the abdominal cavity, which they filled completely. They carried double hooks, 76 and 70 µm in length, respectively, and rows of annular spines typical for the infective larval stage of sebekiids (Riley 1986). The fulcra measured 163 and 153 µm, respectively. The overall length of the mouth was 142 and 161 µm, respectively, and the cadres were 97 and 107 µm long and 57 and 66 µm wide, respectively. Annuli numbered 77 and 72, respectively.

DISCUSSION

At 95 d p.i. the body length, the length of the hooks and fulcra, the mouth dimensions and measurements of the copulatory spicules of male *S. wedli* were virtually identical to those of the adult specimens recovered from crocodile 4, 226 d p.i., indicating that the males had almost reached their final size. However, the females recovered from crocodile 1 were only half the size of the patent adults recovered from crocodile 4. We believe that this difference is due to the slow development of the eggs within the uteri, and thus the gradual increase in the size of the parasite.

The life-cycle of *S. wedli* agrees well with the general developmental pattern of pentastomes that use a single vertebrate intermediate host (Nicoli & Nicoli 1966; Riley 1983). When compared to *S. oxycephala* from the South American caiman, the eggs of *S. wedli* develop into infective larvae in a relatively short time in the intermediate host. The former is known to undergo six moults before the infective stage is reached, about 80 d p.i. (Winch & Riley 1986a). We were not able to isolate primary larvae or developmental stages younger than the infective stage out of the guppies. However, the considerably shorter developmental period of *S. wedli* may indicate that the presumed 6–8 larval stages (Riley 1986) are completed in a shorter time.

In the *Sebekia* spp. only the infective nymph is encysted, which is considered a special characteristic of the genus (Winch & Riley 1986a). The infective larvae recovered during our experiment were still free-living and we assume that they had recently moulted to the infective stage. In another study involving natural infections of *O. mossambicus* and *T. rendalli* by *S. wedli*, the majority of the final instars were encysted (64 and 77%, respectively), whereas the remainder occurred free in the intermediate host (Junker *et al.* 1998). This is to be expected, as natural infection is an ongoing process and larvae in any stage of development could be encountered.

Natural infections with the infective larvae of *S. wedli*, either free-living or encapsulated in the swim bladder, cause little visible harm to their host (Junker *et al.* 1998). We ascribe the severe effect of the larvae on the infected guppy to the unfavourable size ratio between parasite and experimental host and also to the fact that the larvae were still free in the abdominal cavity, rather than encysted in the swim bladder.

To date, no data were available regarding the development of crocodile pentastomes in their final hosts. From this study we deduce that copulation in *S. wedli* occurs about 95 d p.i. which is similar to that of the terrestrial reptilian genera *Porocephalus* and *Armillifer*, where copulation occurs 75–86 d p.i. and 106 d p.i., respectively (Riley 1981; Noc & Curasson 1920). Depending on the species, it takes another 110–155 d before the females reach patency (Riley 1986). *S. wedli* also falls well into this range, as patent females were present 131 d after copulation. The fact that copulation occurs when male and female *S. wedli* are of equal size and that the sex ratio, although initially balanced, shifts towards the females has also been observed in other porocephalid genera (Riley & Self 1980; 1982). Riley (1986) ascribes this to the shorter life span of the male pentastomes. Adult *S. wedli* from experimental hosts possessed all the characteristics typical for this species. The slight difference in size compared to *S. wedli* from natural infections may be due to differences in size between our experimental hosts (<1 m) and the wild-caught hosts (>3m) that we have examined (Junker 1996).

The ability of infective larvae and adult *S. wedli* to survive outside their respective hosts, differs markedly. We ascribe this to the different requirements that the two developmental stages have to meet. Once attached to the respiratory tract of their final host, adult pentastomes live in a fairly constant envi ronment, with high levels of oxygen. When removed from this environment, the parasites die in a matter of hours (Junker 1996). Infective larvae, however, have to make the transition from the fish intermediate host to the crocodilian final host. While infection of the final host is initially a passive process, it subsequently requires a high amount of activity on the part of the parasite to escape from the fish, migrate through its flesh and finally penetrate the stomach or intestinal wall of the crocodile. Once in the final host the infective larvae have to adapt to different osmololarities and physiological conditions and start migrating to the lungs. This requires much tolerance to environmental changes and indicates a high degree of adaptability.

ACKNOWLEDGEMENTS

We thank the Board of Trustees, South African National Parks Board, for placing the animals at our disposal. Our special thanks go to Dr L.E.O. Braack, Kruger National Park, for his help with organization and logistics and Dr John Riley, University of Dundee, Scotland, for his interest in and assistance with this study.

REFERENCES

JUNKER, K. 1996. Untersuchungen zur Biologie und Lebensweise der in Crocodilia parasitierenden Pentastomida in Südafrika. M.Sc. Thesis. University Fridericiana of Karlsruhe.

JUNKER, K., BOOMKER, J. & BOOYSE, D.G. 1998. Pentastomid infections in cichlid fishes in the Kruger National Park, and description of the infective larva of *Subtriquetra rileyi* n. sp. *Onderstepoort Journal of Veterinary Research*, 65:159–167.

NICOLI, R.M. & NICOLI, J. 1966. Biologie des Pentastomides. *Annales de Parasitologie*, 41:255–277.

NOC, F. & CURASSON, G. 1920. Contribution á l'étude de l'évolution biologique de *Porocephalus armillatus* Wyman. *Bulletin de la Société de Pathologie Exotique*, 13:656–659.

RILEY, J. 1981. Some observations on the development of *Porocephalus crotali* (Pentastomida: Porocephalida) in the western diamondback rattlesnake (*Crotalus atrox*). *International Journal for Parasitology*, 11:127–131.

RILEY, J. 1983. Recent advances in our understanding of pentastomid reproductive biology. *Parasitology*, 86:59–83.

RILEY, J. 1986. The biology of pentastomids. *Advances in Parasitology*, 25:45–128.

RILEY, J. & SELF, J.T. 1980. On the systematics and life-cycle of the pentastomid genus *Kiricephalus* Sambon, 1922 with descriptions of three new species. *Systematic Parasitology*, 1:127–140.

RILEY, J. & SELF, J.T. 1982. A revision of the pentastomid genus *Parasambonia* Stunkard and Gandal, 1968; a new generic character, a description of the male, and a new species. *Systematic Parasitology*, 4:125–133.

RILEY, J., SPRATT, D.M. & WINCH, J.M. 1990. A revision of the genus *Sebekia* Sambon, 1922 (Pentastomida) from crocodilians with descriptions of five new species. *Systematic Parasitology*, 16:1–25.

VARGAS, M.V. 1975. Descripcion del huevecillo, larva y ninfa de *Subtriquetra subtriquetra* Sambon, 1922 (Pentastomida), y algunas observaciones sobre su ciclo de vida. *Revista di biologia Tropicale*, 23:67–75.

WINCH, J. M. & RILEY, J. 1986a. Morphogenesis of larval *Sebekia oxycephala* (Pentastomida) from a South American crocodilian (*Caiman sclerops*) in experimentally infected fish. *Zeitschrift für Parasitenkunde*, 72:251–264.

WINCH, J.M. & RILEY, J. 1986b. Studies on the behaviour, and development in fish, of *Subtriquetra subtriquetra*: a uniquely free-living pentastomid larva from a crocodilian. *Parasitology*, 93:81–98.