

Oribatid mites (Acari, Oribatida) as intermediate hosts of tapeworms of the Family Anoplocephalidae (Cestoda) and the transmission of *Moniezia expansa* cysticercoids in South Africa

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ABSTRACT

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Six species of adult oribatid mites (*Galumna racilis*, *Kilimabates pilosus*, *Kilimabates* sp., *Schelorbitates fusifer*, *Muliercula ngoyensis* and *Zygoribatula undulata*) and two immature stages belonging to the superfamilies Galumnoidea and Ceratozetoidea were isolated from a lawn (mixed *Pennisetum* and *Cynodon* spp.) at Onderstepoort Veterinary Institute, South Africa. The mites were subsequently used in an infection trial using *Moniezia expansa* eggs. Tapeworm cysticercoids were recovered in *G. racilis*, *K. pilosus*, *Kilimabates* sp., *S. fusifer*, *M. ngoyensis* and *Z. undulata*, as well as in immatures of Ceratozetoidea. The percentage of infected mites was 7,6, 6,3, 16,4, 66,7, 57,1, 60,0 and 46,7% respectively. Immatures of Galumnoidea did not become infected. The highest number of cysticercoids isolated from one individual was six from an adult *S. fusifer*.

Keywords: Intermediate hosts, *Moniezia expansa*, oribatid mites, transmission of parasites

INTRODUCTION

The cestode family Anoplocephalidae consists of more than 165 species of which the following species are of veterinary importance: *Anoplocephala magna*, *A. perfoliata*, *Anoplocephaloides mamillana*, *Moniezia expansa*, *M. benedeni*, *Avitellina centripunctata*, *Stilesia globipunctata*, *S. hepatica*, *Thysaniezia ovilla* and *Thysanosoma actinoides*. (Hiepe, Buchwalder & Nickel 1985; Denegri, Bernardina, Perez-Serrano & Rodriguez-Caabeiro 1998).

The involvement of oribatid or “beetle” mites in the life cycle of anoplocephalids was shown for the first time by Stunkard (1937). Stunkard experimentally infected *Galumna* mites with eggs of *M. expansa* and observed the development of larval tapeworm stages in their body cavities. The life cycle was then completed by feeding the cysticercoids to a helminth-free raised lamb.

A literature survey made by Denegri (1993) showed that 127 oribatid species belonging to 27 families are known to serve as intermediate hosts to 27 anoplocephalid tapeworm species.

Little is known regarding the role of oribatid mites as transmitters of anoplocephalid tapeworms in Africa. The only study on this aspect of the mites in Africa was made by Graber & Gruvel (1969) in Tchad.

The objective of this study is to determine which species of oribatid mites commonly occurring in cultivated grassland, are able to transmit anoplocephalid cestodes as well as the duration and success rate of transmission.

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MATERIALS AND METHODS

Isolation of oribatid mites

In order to exclude spontaneous infections, oribatid mites were collected from grass samples from an ungrazed lawn (mixed *Pennisetum* and *Cynodon* spp.) at the Theiler Guesthouse of the Onderstepoort Veterinary Institute, South Africa. Since the trial was carried out in the dry winter season in August 1998, the lawn was irrigated 2 d prior to sampling in order to stimulate mites to emerge from the soil and enter the vegetation. Grass samples were cut in the early morning using scissors and transferred to a Berlese funnel, placed in a sun-lit situation (Fig. 1). A Petri-dish filled with tap water, placed at the funnel exit, acted as a trap for mites exiting from the grass cuttings.

The Petri-dish was examined for mites twice daily using a stereo microscope. Mites floating on the water surface were transferred to a moisture cham-

ber, containing moistened filter paper discs, but without food. The moisture chamber was then stored at 4 °C in a refrigerator. Examination of the grass cuttings in the funnel showed that all mites had left the grass sample within two days.

To determine the species of mites, temporary mounts were made on cavity slides with glycerine and examined using a compound light microscope.

Scanning electron microscopy

Ten individuals of each mite species were placed into fine meshed nylon SEM preparation containers (Electron Microscopy Sciences, Fort Washington, PA 19034, USA) and sealed. The containers were immersed in boiling water to heat kill the mites after which the containers were inserted into the cylinder of a 10 cm³ syringe. Treatment solutions were drawn up into the syringe and after the relevant treatment period, expelled again.

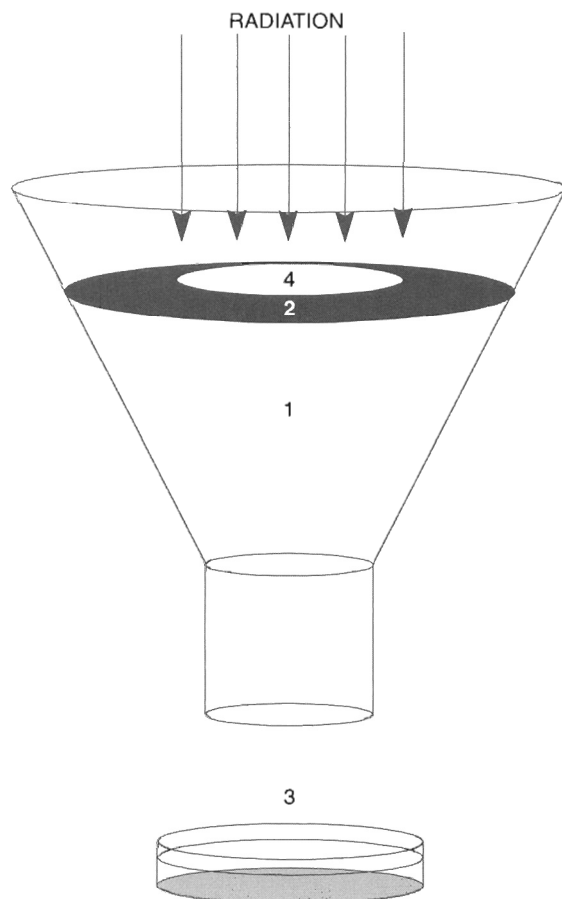


FIG. 1 Scheme of a Berlese funnel for collecting oribatid mites.

1. Funnel
2. Sieve
3. Petri-dish with water
4. Substrate (grass sample)

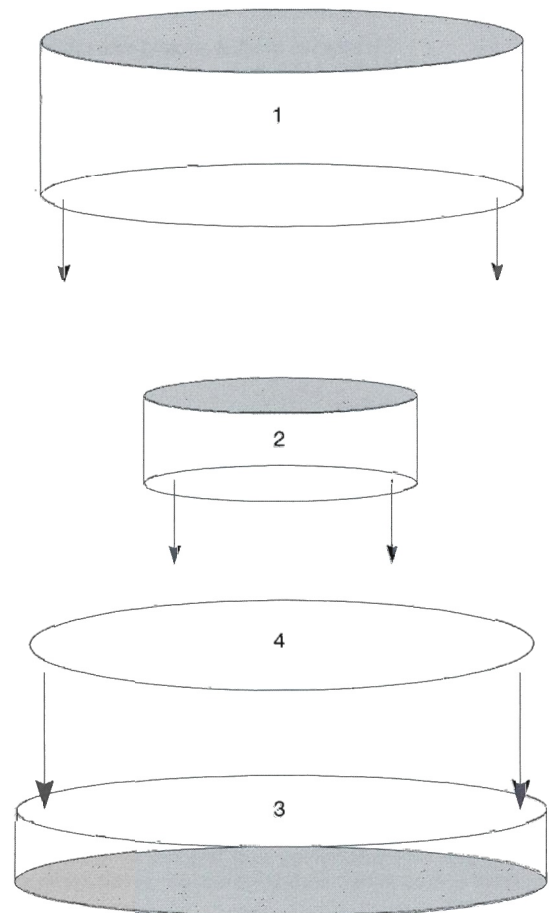


FIG. 2 Scheme of incubation vessels for oribatid mites.

1. Base of large dish
2. Base of small dish
3. Lid of large dish
4. Filter paper

Specimens were washed twice in a saturated detergent solution (Krugel & Du Preez 1995) for 90 min in each treatment and rinsed five times in sterile double distilled water. Samples were dehydrated through an ascending series of ethanols (50, 70, 90, 95, and 3 X 100%—90 min/step) and then critical point dried from 100% ethanol through carbon dioxide. Specimens were mounted individually onto conical brass viewing stubs. Samples were finally cleaned with a fine single-haired brush and sputter coated with gold. Mites were viewed individually at an acceleration voltage of 3–8 kV using an Hitachi S-2500 Scanning Electron Microscope (Hitachi, Tokyo, Japan).

Experimental infection

In three simultaneous experiments, a suspension of *M. expansa* eggs ($n = \pm 10,000$), obtained from terminal segments of tapeworm strobilae removed from the small intestine of a naturally infected lamb, was placed onto a disc of moistened filter paper in a Petri-dish. Starved mites were then added to the Petri-dish and remained in contact with the tapeworm eggs for one week at room temperature. After this period, 100–150 specimens of each mite species were transferred onto another filter paper disc and placed in the lid of a large Petri-dish (Fig. 2). Mites were restricted to the centre of the filter paper disc by covering them with a smaller lid of another Petri-dish. Humidity was regulated by periodically adding water to the filter paper. The whole system was covered by the base of the large Petri-dish and stored in an incubator at 28 °C for four weeks. Pieces of fresh potato and humus from the collection site served as food for the mites. Every third day the system was removed from the incubator and opened for airing at room temperature for ten min, during which time dead mites were removed.

Examination of mites for cysticercoids

Starting on the 25th day of incubation, mites were removed from the moisture chamber and dissected under a stereo microscope. They were transferred to a drop of water on a slide and crushed using a dissecting lancet. The content of the body cavity was removed with dissecting needles and examined under a light microscope using the 40 X objective.

RESULTS

Among the oribatid fauna of the Onderstepoort Veterinary Institute, representatives of the following six species were found as adults on a lawn viz., *Galumna racilis* Pérez-Iñigo, 1987; *Kilimabates pilosus* Mahunka, 1985; *Kilimabates* sp., *Scheloribates fusifer* Berlese, 1908; *Muliercula ngoyensis* Coetzer, 1968 and *Zygoribatula undulata* Berlese, 1917. An addi-

tional two forms of immature mites were found to be Galumnoidea and Ceratozetoidea species, probably *G. racilis* and *Kilimabates* sp. respectively.

Adults of *G. racilis*, *Kilimabates* sp. and *Z. undulata*, as well as immatures of Ceratozetoidea were present in high numbers, while *K. pilosus*, *S. fusifer* and *M. ngoyensis* occurred in moderate densities, and only a few specimens of immature Galumnoidea were isolated from the grass samples.

The highest percentage of infection of mites exposed to *M. expansa* eggs was found in *S. fusifer*, *Z. undulata*, *M. ngoyensis* and the immature Ceratozetoidea (Table 1). Most infected mites contained low numbers of tapeworm larvae, with the highest burden of six cysticercoids being removed from a specimen of *S. fusifer* (Table 2). At day 25 after incubation, it was found that all positive mites still harboured tapeworm larvae in the elongated invagination stage, while fully developed cysticercoids (Fig. 3) were only found 28 d after incubation. The infected mites did not contain any mite eggs, while low numbers of mite eggs (one to three) were found in only one third of the adult mites which were negative for cysticercoids.

DISCUSSION

This is the first experimental observation of the intermediate hosts of *M. expansa* in South Africa. Data reveal for the first time that *G. racilis*, *K. pilosus*, *Kilimabates* sp., *S. fusifer*, *M. ngoyensis* and *Z. undulata* can act as intermediate hosts for this tapeworm species. Oribatid mites belong to the normal soil fauna of natural terrestrial biotopes or grass lands.

TABLE 1 Percentage of experimentally infected oribatid mites positive for *M. expansa* cysticercoids

Mite species	Number of mites examined	Number of mites with cysticercoids	Percentage of positive mites
<i>Galumna racilis</i>	53	4	7,55
Galumnoidea immatures	28	0	0,0
<i>Kilimabates pilosus</i>	48	3	6,25
<i>Kilimabates</i> sp.	55	9	16,36
Ceratozetoidea immatures	45	21	46,66
<i>Scheloribates fusifer</i>	42	28	66,66
<i>Muliercula ngoyensis</i>	35	20	57,14
<i>Zygoribatula undulata</i>	35	21	60,00

TABLE 2 Number of *M. expansa* cysticercoids removed from the body cavity of oribatid mites after artificial infection

Mite species	Number of cysticercoids per mite						
	0	1	2	3	4	5	6
<i>Galumna racilis</i>	49	2	1	1	0	0	0
Galumnoidea immatures	28	0	0	0	0	0	0
<i>Kilimabates pilosus</i>	45	2	1	0	0	0	0
<i>Kilimabates</i> sp.	46	5	4	0	2	0	0
Ceratozetoidea immatures	24	16	4	1	0	0	0
<i>Scheloribates fusifer</i>	14	10	4	6	5	2	1
<i>Muliercula ngoyensis</i>	15	10	9	1	0	0	0
<i>Zygoribatula undulata</i>	14	14	4	3	0	0	0



FIG. 3 Fully developed *Moniezia expansa* cysticercoid removed from oribatid mites

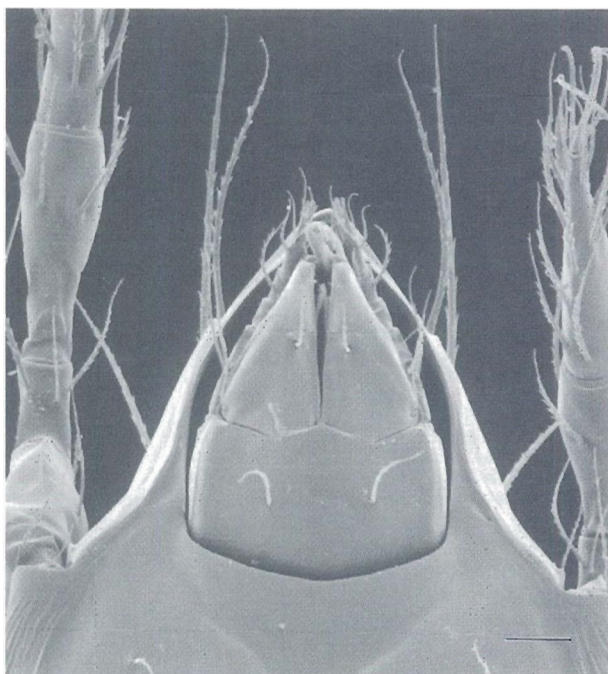


FIG. 4 Gnathosoma of *Scheloribates fusifer* (500 X)

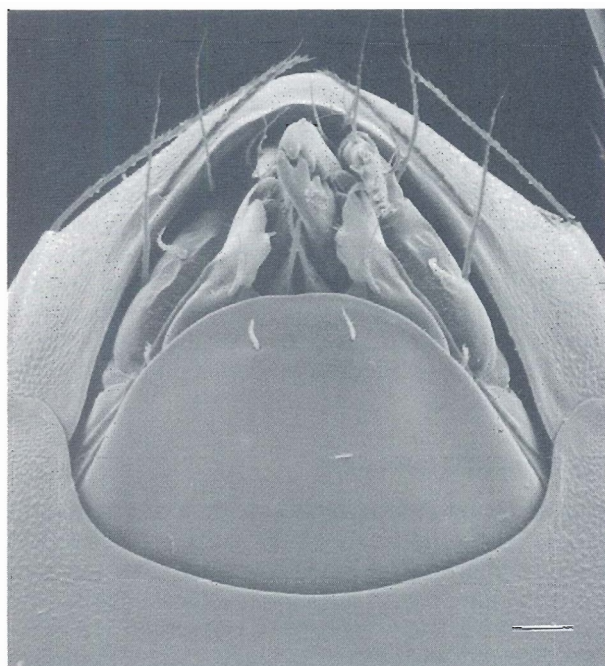


FIG. 5 Gnathosoma of *Galumna racilis* (500 X)

They are absent or occur only in low numbers in cultivated fields and gardens (Weigmann 1984; Skubala & Dziuba 1995). The epidemiological role of various oribatid mite species is determined by their susceptibility to tapeworm eggs and the natural occurrence of mites on pastures.

According to Saldybina (1953) and Kassai & Mahunka (1965), the body size, the size of the mouth opening and the structure of the mouth-parts are the main attributes of an oribatid mite enabling it to act as an intermediate host for anoplocephalid cestodes. Oribatid mites can only become infected when they mechanically destroy the outer shell of the tapeworm eggs (Caley 1975), while larger mites are able to swallow tapeworm eggs whole (Fig. 4, 5, 6, 7). In the latter case the oncosphera are not able to hatch and the egg passes through the alimentary tract of the mite (Ebermann 1976). This may explain the lower percentage of infected specimens in the larger mites in this study, viz., *Galumna* and *Kilimabates*. Fig. 4 and 5 show the difference in size of the gnathosoma between *S. fusifer* (Fig. 4) and *G. racilis* (Fig. 5). Fig. 5 and 6 show the strongly armoured mouthparts of *S. fusifer*.

The prevalence of infected mites under natural conditions is usually low and does not exceed 3% (Prokopic 1962; Ejtminavcjute 1963; Bankov 1965; Kassai & Mahunka 1965; Al'kov 1972; Skorski, Barutzki & Boch 1984; Barutzki & Parwar 1986; Schuster 1988) while under experimental conditions, up to 90% of mites may become infected (Schuster 1988). Since the development of cysticercoids within

FIG. 6 Rutella and chelicerae of *Schelorbates fusifer* (2500 X)FIG. 7 Chelicera of *Schelorbates fusifer* (4000 X)

the intermediate host is a volume demanding process, the number of cysticercoids per mite is limited and usually does not exceed one to three cysticercoids/larvae per individual. More than ten larvae per mite are seldom found (Jurasek 1962).

Our own investigations confirmed results of previous studies that infected mites usually do not carry mite eggs (Schuster 1995) or have considerably fewer eggs compared to uninfected control specimens (Trowe 1997). However, due to the low infection rate of oribatids under natural conditions, the presence of the parasite and its sterilizing effect on the mites, does not influence the mite population. The low fertility rate in the current study could be the result of unfavourably dry conditions at the time of collection.

In the literature cited, cysticercoids were established in adult oribatid mites and only Narsapur (1978) reported the development of *Moniezia* larval stages in immature mites. It was noted that only the nymphal stages of *Platynothrus peltifer* (Koch 1839) could be infected and not the adult mites. In our own investigations immature stages of Ceratozetoidea showed a much higher cysticercoid prevalence than the adult stages (genus *Kilimabates*). This might be explained by a lower susceptibility of adult mites in this group.

The developmental duration of cysticercoids is mainly temperature dependant. Narsapur & Prokopic (1979) suggested that, for experimental purposes, it was necessary to incubate infected mites at 28 °C. Under these conditions, infective cysticercoids are available at day 27. Our own investigations con-

firmed these data and showed that, if incubated at 28 °C, mites still had undeveloped tapeworm larvae at day 25, while fully developed cysticercoids occurred on d 28. Under natural conditions, and at temperatures between 16–20 °C, the developmental duration is approximately three months (Kuznecov 1966). Epidemiological studies on the prevalence of moniezirosis in final hosts in Europe showed seasonal variations. *Moniezia expansa* has a high prevalence in spring (April to June) while *M. benedeni* causes moniezirosis in autumn (September to November). According to Andreeva & Akbaev (1993), the infection peak of final hosts with anoplocephalid cestodes depends on the activity of the mites and their presence in grass after rainfalls in spring and autumn. Van Nieuwenhuizen, Verster, Horak, Krecek & Grimbeek (1994) concluded that soil moisture and temperature interact to increase the relative humidity in the canopy of the vegetation. Apparently this stimulates the mites to migrate from the soil to the vegetation during summer when soil moisture is high. However, they found a significant higher number of mites on the vegetation in the early morning than during the rest of the day, and suggested that the mites migrate back into the soil to escape the unfavourable conditions of midday. Despite irrigation during dry periods, there is an increase in the number of mites during summer, when rainfall is high (Van Nieuwenhuizen *et al.* 1994) and soil moisture seems to be one of the most important environmental factors regulating the vertical movement of the mites (Metz 1971; Sheela & Haq 1991). The risk of infection for the final host is therefore greatest during

months of high rainfall and early in the morning, decreasing as the day becomes warmer.

It seems as if there is a whole suite of factors influencing the success of the various species of oribatid mites as intermediate hosts of *Moniezia*, e.g. size of the mite (Sengbush 1977; Xiao & Herd 1992), structure of the mouthparts (Caley 1975), habitat preferences and behaviour of the mites (Wallwork & Rodriguez 1961). This study indicates that under local conditions in South Africa, the Oripodoidea (*Schelorbates fusifer*, *Muliercula ngoyensis*, *Zygoribatula undulata*) is the most successful in terms of susceptibility to the cysticercoids, although they are not necessarily the most abundant.

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