

**A COCCIDIAN IN HAEMOGAMASID MITES;  
POSSIBLE VECTORS OF *ELLEIPSISOMA THOMSONI* FRANCA, 1912**

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**SUMMARY.** Haemogamasinae/Laelapidae mites (*Haemogamasus hirsutus*, *H. nidi* and *Eulaelaps stabularis*) collected from nests of the mole (*Talpa europaea*) contained developmental stages (isosporean-type oocysts and independently developing macro-and microgametocytes) of a coccidian. These stages were observed in the haemocoel of living infected mites, in wet preparations of crushed mites, and in histological sections of paraffin wax embedded mites. They included both unsporulated and sporulated oocysts; sporulation of the oocysts occurred within the mite. Descriptions of the sporogonic and gametogonic stages of this coccidian are given and compared with the suborders Adeleina and Eimeriina which either have developmental stages in invertebrates, isosporean-type oocysts or have been reported to be mechanically (passively) transmitted by mites. The possibility of the haemogamasid mites being the vector of *Elleipsisoma thomsoni* or other coccidian parasites is also discussed.

*Key-words:* Coccidian. Haemogamasid mites. *Elleipsisoma*. *Talpa*.

**Une Coccidie chez des Acariens gamasides, vecteurs éventuels d'*Elleipsisoma thomsoni* Franca, 1912.**

**RÉSUMÉ.** Les Acariens *Haemogamasus hirsutus*, *H. nidi* et *Eulaelaps stabularis* (Laelapidae, Haemogamasinae) récoltés dans les nids de la taupe (*Talpa europaea*), présentent différents stades de développement d'une Coccidie, d'une part oocystes de type *Isospora*, d'autre part macro- et microgamétocytes en cours de développement. Ces stades sont décrits à partir de l'hémacoel d'Acariens vivants, de frottis par apposition d'Acariens écrasés et de coupes histologiques. Ils comprennent des oocystes sporulés et non sporulés. La sporulation des oocystes a donc lieu chez l'Acarien. Les stades de la gamétogonie et de la sporogonie de cette Coccidie sont décrits et comparés avec ceux des Adeleina et Eimeriina, connus pour se développer avec des oocystes de type *Isospora* chez les Invertébrés, ou qui ont été signalés comme étant transmis mécaniquement (passivement) par des Acariens. Le rôle des Gamasides comme vecteur d'*Elleipsisoma thomsoni* ou d'autres Coccidies est discuté.

*Mots-clés :* Coccidies. Acariens Gamasides. *Elleipsisoma*. *Talpa*.

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## Introduction

Developmental stages (of sporogony and gametogony) of a coccidian were observed in Haemogamasinae/Laelapidae mites collected from nests of the mole (*Talpa europaea*). In the sporogonic stage, isosporan-type oocysts (both unsporulated and sporulated) were seen, while in the gametogonic stage, independently developing macro- and microgametocytes were observed.

In the moles (*Talpa europaea*) with which the haemogamasid mites were associated and from whose nests they were collected, *Elleipsisoma thomsoni* Franca (1912), an apicomplexan parasite in the red blood cells of the mole was reported (Mohamed & Molyneux, 1984). In the life cycle of *E. thomsoni* only the stage in the red blood cells (with merogony in the red blood cells in the lungs) is definitely known (Franca, 1912).

This paper presents the first descriptions of the sporogonic and gametogonic stages of the coccidian in the mite, discusses the possibility of the stages of the coccidian in the mite being those of *E. thomsoni* and the potential role of mites as vectors.

## Materials and methods

### MITES

Mites Haemogamasinae/Laelapidae were collected by hand or Berlese funnel from nests of the mole (*Talpa europaea*). They were examined under the light microscope either intact or crushed in normal saline. Samples of the infected mites were kindly identified by Professor A. Fain, Institut Royal des Sciences Naturelles de Belgique, Antwerp. The infected mites all belonged to the subfamily Haemogamasinae Oudemans, 1926, family Laelapidae Berlese 1892 and were the following species:

*Haemogamasus hirsutus* (Berlese, 1889).

*Haemogamasus nidi* (Michael 1892).

*Eulaelaps stabularis* (Koch, 1836).

### HISTOLOGICAL SECTIONS

Samples of mites from the nests found to contain infected mites were processed for histology. They were paraffin wax embedded and section 5-10  $\mu\text{m}$  thick were cut, stained with Giemsa-Colophonium (Bray and Garnham, 1962) and examined under the light microscope (Leitz, Dialux).

## MEASUREMENT OF PARASITES

The measurement of oocysts in the wet preparation and the gametocytes in the stained histological sections were made with a calibrated ocular micrometer (mag  $\times$  250).

## INFECTIVITY

About 0.1-0.2 ml of normal saline containing sporulated oocysts, from the mites, was inoculated intraperitoneally into each of 4 mice (BK/SWR strain) and 6 field voles (laboratory-bred, 4 week-old, *Microtus agrestis*); before inoculation all animals were checked for coccidia in their faeces and were found to be negative. Inoculated animals were checked daily for 6 weeks by wet preparations and Giemsa-stained smears of the tail blood and by faecal examinations.

## Results

## WET PREPARATIONS

Of 133 mites (*H. hirsutus*, *H. nidi* and *E. stabularis*) crushed in normal saline, 10 (7.5 %) were found infected with a coccidian; oocysts (both unsporulated and sporulated) were present in all three mite species.

The oocysts were of one particular type which was elliptical in shape, surrounded with a smooth double-layered wall, without a micropyle, polar granule or oocyst residuum. The oocysts measured  $30-35 \times 25-30 \mu\text{m}$  (mean  $33.85 \times 28.45 \mu\text{m}$ ;  $\pm 2.08 \times \pm 2.24$ ) (n. = 20) and contained a granular cytoplasm which filled the whole volume of the oocyst (*fig. 1a*). Sporulation of the oocyst took place within the mite and oocysts with two sporoblasts or others with two sporocysts were observed (*fig. 1, b-c*).

The fully sporulated oocyst contained two sporocysts, each with four sporozoites (*fig. 1, d-f*). The sporocysts were ellipsoidal with no stieda or substiedal bodies but with a granular sporocyst residuum. The sporocysts measured  $27-29 \times 14-20 \mu\text{m}$  (mean  $27.5 \times 14.5 \mu\text{m}$ ;  $\pm 1.27 \times \pm 1.70$ ) (n. = 20). The sporozoites were banana-shaped, slightly tapering at one end (*fig. 1, d (inset)*) and measured  $15-18 \times 4-5 \mu\text{m}$  (mean  $16.0 \times 4.7 \mu\text{m}$ ;  $\pm 1.6 \times \pm 0.41$ ) (n. = 10).

Observations of living infected mites under saline revealed oocysts to be free and circulating in the haemocoelic fluid (*fig. 2*).

## HISTOLOGICAL SECTIONS

In histological sections of the mites, both gametogony and sporogony of this parasite were seen.

The macro- and microgametocytes developed separately in the haemocoel and in the fat cells of the mite (*fig. 3*). The macrogametocytes were spherical and measured  $20-25 \times 15-20 \mu\text{m}$  (mean  $20.2 \times 16.0 \mu\text{m}$ ;  $\pm 1.0 \times \pm 1.2$ ) (n. = 10). They had

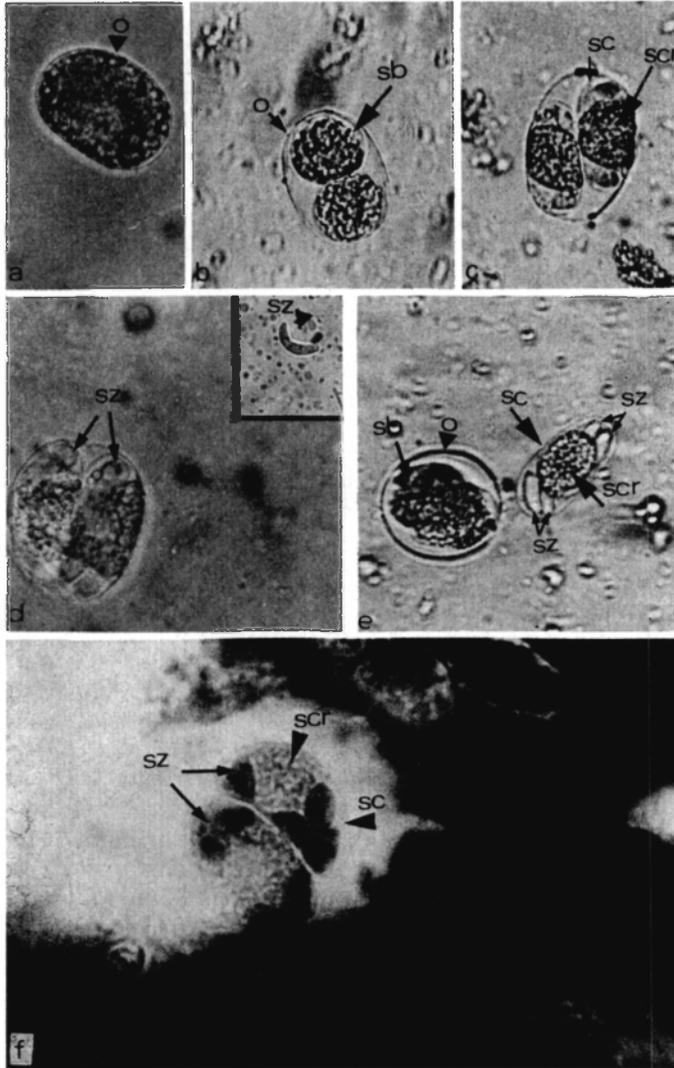


FIG. 1. — Various developmental stages of the isosporan-type oocyst of the coccidian found in the haemogamasinae mites. All stages of sporulation in this figure were seen within the mite. Magnification a-e  $\times 1,250$ .

- a) An unsporulated oocyst (o) found in the wet preparation; note the absence of both micropyle and polar granule.
- b) A sporulating oocyst (o) showing two sporoblasts (sb).
- c) A sporulated oocyst showing two sporocysts (sc) each with its own sporocyst residuum (scr); note the absence of an oocyst residuum.
- d) A sporulated oocyst with two sporocysts showing the sporozoites (sz). Inset: a free sporozoite (sz); note the banana-shaped structure of the sporozoite.
- e) A sporulating oocyst (o) and a separate sporocyst (sc) showing four sporozoites (sz) and a sporocyst residuum (scr).
- f) A histological section of a mite showing a sporulated oocyst with two sporocysts (sc), each with four sporozoites (sz); note also the sporocyst residuum (scr). (Mag  $\times 2,250$ ).

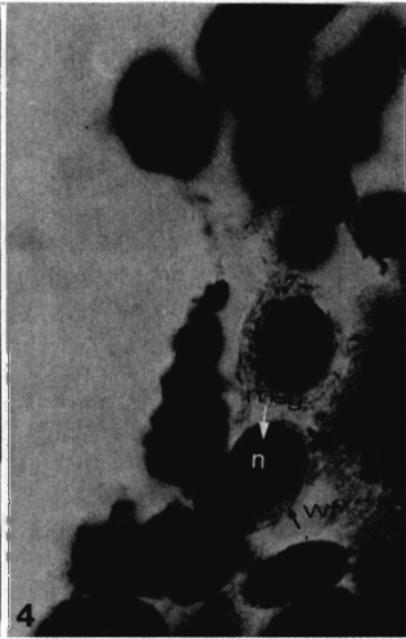
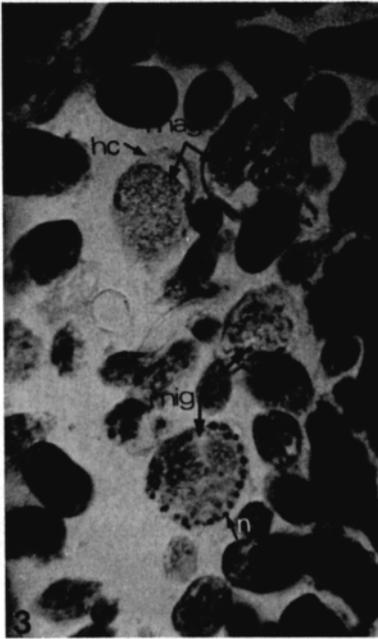
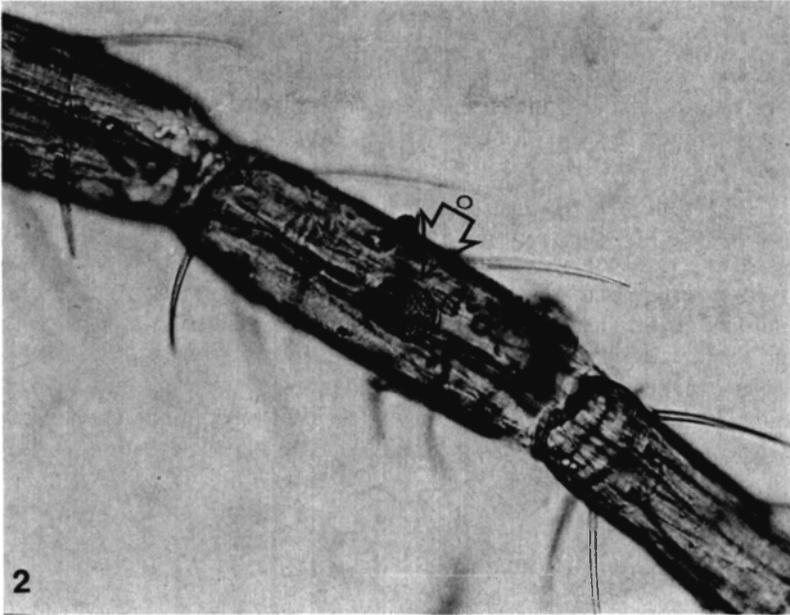


FIG. 2. — A mite leg showing an oocyst (o) free in the haemocoelic fluid of a living mite (Mag.  $\times 600$ ).

FIG. 3. — A histological section of a mite showing macro- (mag) and microgametocytes (mig). Note the parasitized host cell (hc) and the peripheral arrangement of the nuclei (n) in the developing microgametocyte (Mag.  $\times 1,640$ ).

FIG. 4. — A macrogametocyte (mag) showing a central nucleus (n) with nucleolus. The dark granules (wf) in the cytoplasm of the macrogametocyte are the wall-forming bodies (Mag.  $\times 1,640$ ).

a single centrally located nucleus and the cytoplasm contained various cellular elements including the wall-forming bodies which were more easily seen in the mature macrogametocytes (*fig. 4*).

The mature microgametocytes were mostly ovoid to ellipsoidal and measured  $20\text{--}25 \times 15\text{--}20 \mu\text{m}$  (mean  $21.5 \times 17.5 \mu\text{m}$ ;  $\pm 0.71 \times \pm 0.8$ ) ( $n = 6$ ). In the immature microgametocytes there were many fragmented nuclei in the cytoplasm (*fig. 5*) which subsequently became spherical or elliptical in the mature microgametocytes and gradually oriented towards the periphery of the limiting membrane (*fig. 6*), forming the nuclei of many developing microgametes.

Histological sections also revealed oocysts (both unsporulated and sporulated) developing extra-intestinally in the haemocoel of the mite (*fig. 7*); a few free sporozoites were also observed (*fig. 8*).

#### INFECTIVITY

None of the animals inoculated with the parasites in the normal saline became infected.

### Discussion

Mites, ticks, sucking lice, fleas, various mosquitoes and sandflies have been proven or are suspected as vectors of *Hepatozoon* species (Hoogstraal, 1961). Hoogstraal (*loc. cit.*) reported the development of sporogonic stages of *Hepatozoon balfouri* of jerboas (*Jaculus* spp.), in the mite, *Haemolaelaps aegyptius* while Furman (1966) described both early and late sporogonic stages of that parasite, in the mites, *Haemolaelaps longipes* and *Haemolaelaps centrocarpus*.

The mite *Haemogamasus reidi* (= *Echinolaelaps echidninus*) has been reported to be the invertebrate host of *Hepatozoon muris* of rats (see review by Killick-Kendrick, 1974); all stages of sporogony were seen in the mite. The sporogony of *Hepatozoon griseisciuri* of the grey squirrel has also been described in *Haemogamasus reidi* and in *Euhaemogamasus ambulans* (Clark, 1958; Redington and Jackowski, 1971).

The genera *Hepatozoon*, *Haemogregarina* and *Karyolysus* make up the apicomplexan family Haemogregarinidae of the suborder Adeleina (Levine, 1973a; Levine *et al.*, 1980). In the life-cycle of these genera merogony occurs in the internal organs of a vertebrate host, gametogony in red or white blood cells (also in the vertebrate host) and sporogony in an invertebrate (Landau, 1973). Sporulation of the genera *Hepatozoon* and *Karyolysus* takes place in a large cyst forming numerous sporocysts, while in the genus *Haemogregarina* small oocysts produce sporozoites without sporocysts (Ball, 1967; Manwell, 1977; Nadler & Miller, 1984). The macro- and microgametocytes of these genera are usually associated in syzygy (*i. e.* they lie up against each other) during development (Levine *et al.*, 1980) and microgametocytes produce 1-4 microgametes.

The parasite seen in the mites (*H. hirsutus*, *H. nidi*, *E. stabularis*) from moles

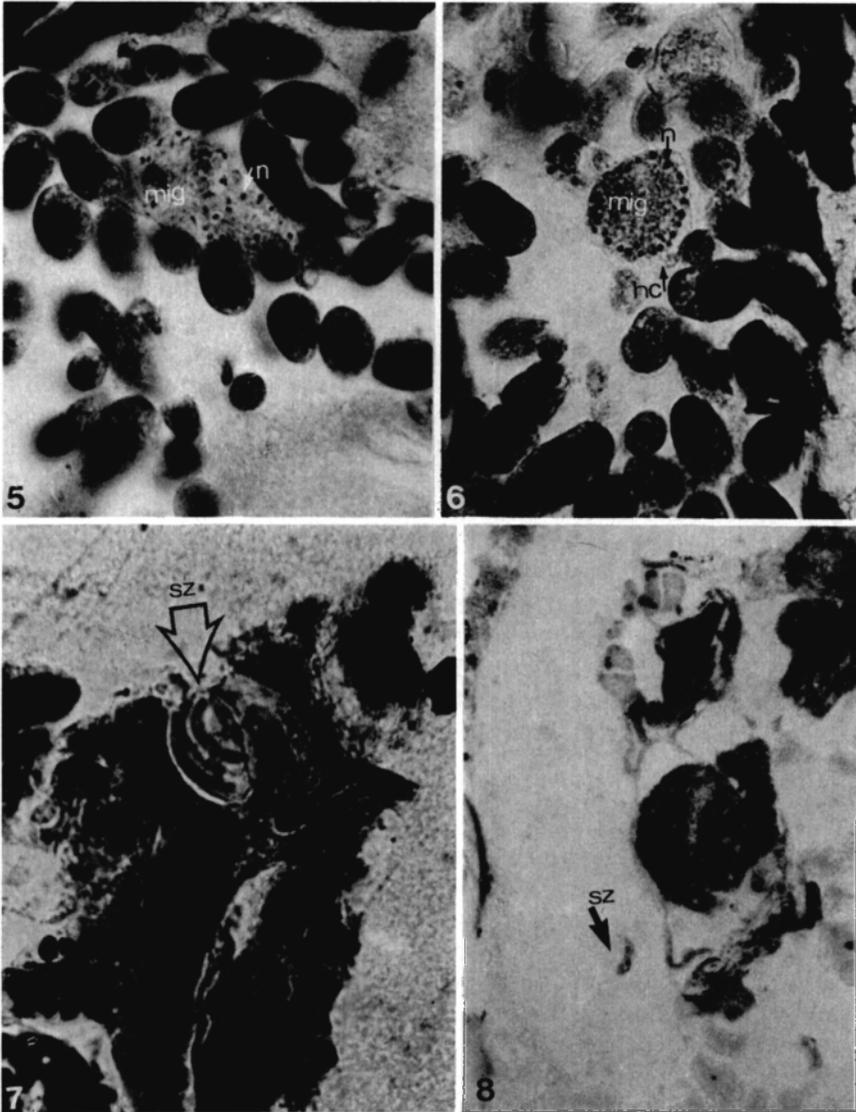


FIG. 5. — An immature microgametocyte (mig) showing the division of the nuclei (n). (Mag.  $\times 1,640$ ).

FIG. 6. — A microgametocyte (mig) at an advanced stage of development showing the peripheral arrangement of the nuclei (n); note also the parasitized host cell (hc). (Mag.  $\times 1,640$ ).

FIG. 7. — A histological section of the mite haemocoel showing part of a sporulated oocyst with some sporozoites (sz). (Mag.  $\times 1,640$ ).

FIG. 8. — A free sporozoite (sz) in the mite haemocoel. (Mag.  $\times 625$ ).

is clearly different from the above genera (*Hepatozoon*, *Haemogregarina*, *Karyolysus*) on the basis of its sporogony (oocysts produce two sporocysts, each with four sporozoites) and gametogony (macro- and microgametocytes develop independently; microgametocytes produce many microgametes).

In the suborder Eimeriina, of the phylum Apicomplexa, Levine (1973b) reported that only the genera *Isospora* and *Toxoplasma* (together with related genera of the family Sarcocystidae: *Sarcocystis*, *Frenkelia*, *Hammondia*, *Besnoitia*, *Cystoisospora*) have two sporocysts with four sporozoites each, as are seen in this parasite.

*Isospora* parasites are normally homoxenous occurring in the intestine of the vertebrate host; *Toxoplasma* (and related genera) are heteroxenous (or sometimes homoxenous) occurring in the tissue of one host and the intestine of the other (or sometimes in both the tissue and intestine of the same animal) (Levine, 1978). To date, neither *Isospora* nor *Toxoplasma* (and related genera) have been reported to be transmitted or to develop in invertebrate hosts (see review by Frenkel, 1974). The oocysts and sporocysts of *Isospora* species measure  $12-43 \times 10-33 \mu\text{m}$  and  $9-27 \times 6-24 \mu\text{m}$  respectively; those of *Toxoplasma* (and related genera) measure  $11-14 \times 9-11 \mu\text{m}$  for the oocysts and  $8.5-17 \times 6-13 \mu\text{m}$  for the sporocysts (see review by Frenkel, 1974; Frenkel, 1977; Dubey, 1977; Levine, 1978; Tadros & Laarman, 1982; Smith, 1982; Lee, Hutner and Bovee, 1985).

The genera *Lankesterella* and *Schellackia* of the suborder Eimeriina, family Lankesterellidae, which parasitize birds and amphibians are passively transmitted (*i. e.* without development) by invertebrates (mites or leeches) (Levine, 1973b); oocysts produce 32 or more naked sporozoites (without sporocysts) in *Lankesterella* and 8 naked sporozoites in *Schellackia*. Both these genera are clearly different from this parasite.

The parasite, in this study, has an isosporan-type oocyst and sporocysts, which are within the measurement range of the genus *Isospora* but since, to date, none of the known species of *Isospora* have been reported to have developmental stages in an invertebrate it is possible that the gametogony and sporogony of the isosporan-type coccidian seen in the mite are the missing developmental stages of *E. thomsoni*. The stages of *E. thomsoni* in the mole could be the asexual stage of this isosporan-type coccidian which may have extraintestinal development as has been reported in some *Isospora* (Box, 1981).

*E. thomsoni* is clearly different from all known blood parasites of insectivores, rodents and bats (Mohamed & Molyneux, 1984). This is the first report of an isosporan-type coccidian in mites (*H. hirsutus*, *H. nidi* and *E. stabularis*). It is possible that haematophagous mites become infected with *E. thomsoni* from the peripheral blood of moles and that gametogony and sporogony take place in the mite with the transmission of the infective sporozoites to the mole by ingestion (of the mite) or by inoculation (by bite of the mite). If this is the case, *E. thomsoni* has a typical sporozoan life cycle of three-phases (*fig. 9*).

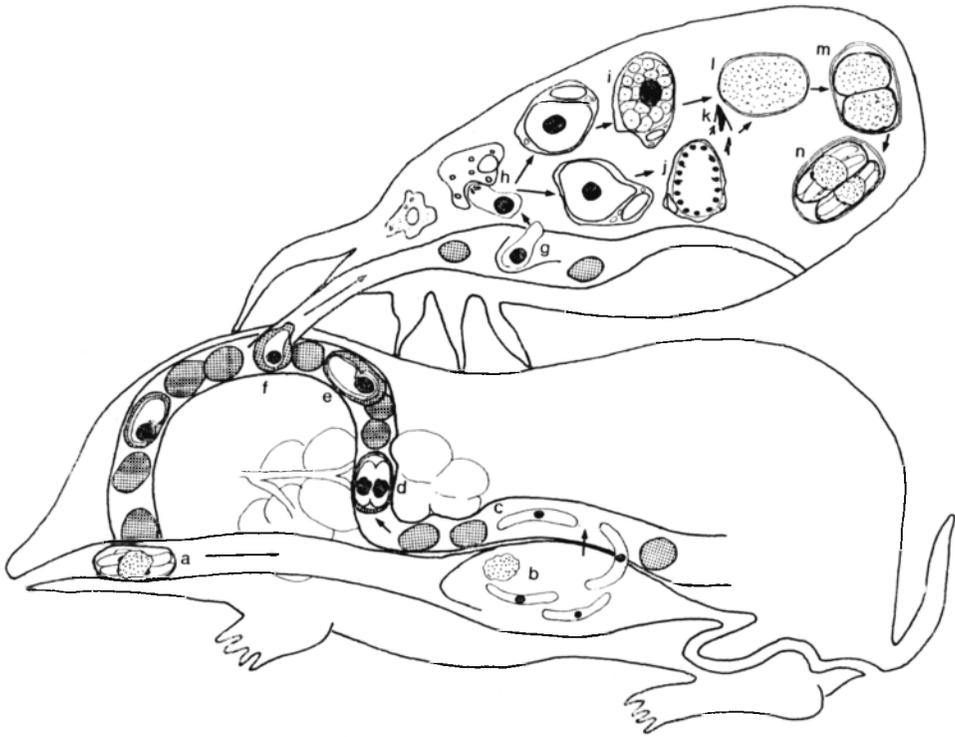


FIG. 9. — Putative life cycle of *E. thomsoni*: Sporocysts (*a*) in infected mites ingested by the mole break open in the gut releasing sporozoites (*b*) which penetrate the gut wall and enter the blood system (*c*). The sporozoites are carried to the lung where merogony occurs (*d*) within erythrocytes. The resulting merozoites (*e*) enter the peripheral blood and are taken up (*f*) by feeding mites. The merozoites then penetrate the gut epithelium of the mite (*g*) enter the haemocoel and invade fat cells (*h*) developing either as macrogametocytes (*i*) or microgametocytes (*j*). Microgametes (*k*) fuse with a mature macrogametocyte and the zygote sporulates (*l, m*), producing oocysts (*n*) with two sporocysts, each with four sporozoites. Diagrammatic and not to scale.

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#### REFERENCES

- BALL G. H. : Some blood sporozoans from East African reptiles. *J. Protozool.*, 1967, 14, 198-210.  
 BERLESE A. : Acari, Myriopoda et Scorpiones Hucusque in Italia Reperta. Patavii, Florentiae, 1882-1892.  
 BOX E. D. : *Isospora* as an extraintestinal parasite of passerine birds. *J. Protozool.*, 1981, 28, 244-246.

- BRAY R. S., GARNHAM P. C. C. : The giemsa-colophonium method for staining protozoa in tissue sections. *Indian J. Malariol.*, 1962, *16*, 153-155.
- CLARK G. M. : *Hepatozoon griseisciuri* n. sp.; a new species of *Hepatozoon* from the grey squirrel (*Sciurus carolinensis* Gmelin, 1788) with studies on the life cycle. *J. Parasitol.*, 1958, *44*, 52-63.
- DUBEY J. P. : *Toxoplasma*, *Hammondia*, *Besnoitia*, *Sarcocystis* and other tissue cyst-forming coccidia of man and animals. In: Parasitic Protozoa (J. P. Kreier, Ed.). *Academic Press*, New York, San Francisco, London, 1977. Vol. III, 101-237.
- FRANCA C. : Sur les hematozoaires des taupes. *Arq. Instit. Bacteriol. Camera Pestana*, 1912, *III*, 271-278.
- FRENKEL J. K. : Advances in the biology of Sporozoa. *Z. Parasitkde.*, 1974, *45*, 125-162.
- FRENKEL J. K. : *Besnoitia wallacei* of cats and rodents: with a reclassification of other cyst forming isosporoid coccidia. *J. Parasitol.*, 1977, *63*, 611-628.
- FURMAN D. P. : *Hepatozoon balfourii* (Laveran 1905) sporogonic cycle, pathogenesis, transmission by mites to jerboa hosts. *J. Parasitol.*, 1966, *52*, 373-382.
- HOOGSTRAAL H. : The life cycle and incidence of *Hepatozoon balfourii* (Laveran 1905) in Egyptian jerboas (*Jaculus* spp.) and mites (*Haemolaelaps aegyptius* Keegan 1956). *J. Protozool.*, 1961, *8*, 231-248.
- KILLICK-KENDRICK R. : Parasitic protozoa of the blood of rodents. II. Haemogregarines. Malaria parasites and piroplasms of rodents: an annotated checklist and host index. *Acta Trop.* (Separatum), 1974, *31*, 28-69.
- KOCH C. L. : Crustaceen, Myriopoden und Arachniden Deutschlands, Regensburg, 1835-1844.
- LANDAU I. : Diversité des mécanismes assurant la pérennité de l'infection chez les sporozoaires coccidiomorphes. *Mem. Mus. Nat. Hist. Nat.*, 1973, A *77*, 1-61.
- LEE J. J., HUTNER S. H., BOVEE E. G. : An illustrated Guide to the Protozoa. Society of Protozoologists. *Allen Press*, Lawrence, Kansas, 1985, p. 629.
- LEVINE N. D. : Protozoan parasites of domestic animals and of man. *Burgess Publishing Company*, Minneapolis (Minnesota), 1973a.
- LEVINE N. D. : Introduction, history and taxonomy. In: The Coccidia (D. M. Hammond, P. L. Long, Eds). *University Park Press*, Baltimore, Butterworths, London, 1973b, 1-22.
- LEVINE N. D. : Textbook of Veterinary Parasitology. *Burgess Publishing Company*, Minneapolis (Minnesota), 1978.
- LEVINE N. D., CORLISS J. O., COX F. E. G., DEROUX G., GRAIN J., HONIGBERG B. M., LEEDALE G. F., LOEBLICH A. R., LOM III-J., LYNN D., MERINFELD E. G., PAGE F. C., POLJANSKY G., SPRAGUE V., VAVRA J., WALLACE F. G. : A newly revised classification of the protozoa. *J. Protozool.*, 1980, *27*, 37-58.
- MANWELL R. D. : Gregarines and Haemogregarines. In: Parasitic Protozoa (J. P. Kreier, Ed.). *Academic Press*, New York, San Francisco, London, 1977, Vol. III, 1-32.
- MICHAEL A. D. : On the variations in the internal anatomy of the Gamasinae. *Trans. Linnean Soc. Lond.*, 1892, *5*, 31-37.
- MOHAMED H. A., MOLYNEUX D. H. : *Elleipsisoma thomsoni* Franca, 1912: an apicomplexan parasite in the red blood cells of the mole (*Talpa europaea*). *Parasitology*, 1984, *89*, 407-415.
- NADLER S. A., MILLER J. H. : A redescription of *Hepatozoon mocassini* (Laveran, 1902) n. comb. from *Agkistrodon piscivorus leucostoma* Troost, 1836. *J. Protozool.*, 1984, *31*, 321-324.
- OUDEMANS A. C. : Acarologische Aanteekeningen. 79. *Entologische Berichten*, Amsterdam, 1926, *7* (146), 26-34.
- REDINGTON B. C., JACKOWSKI L. A. : Syngamy and sporogony of *Hepatozoon griseisciuri* Clark, 1958 (Sporozoa: Haemogregarinidae) in its natural vector, *Haemogamasus reidi* Ewing, 1925 (Acari: Mesostigmata). *J. Parasitol.*, 1971, *57*, 953-960.
- SMITH D. D. : The Sarcocystidae: *Sarcocystis*, *Frenkelia*, *Toxoplasma*, *Besnoitia*, *Hammondia* and *Cystoisospora*. *J. Protozool.*, 1982, *28*, 262-266.
- TADROS W., LAARMAN J. J. : Current concepts on the biology, evolution and taxonomy of tissue-forming Eimeriid coccidia. *Adv. Parasitol.*, 1982, *20*, 293-468.