HEPATOZOOON KISRAE N. SP. INFECTION THE LIZARD AGAMA STELLIO IS TRANSMITTED BY THE TICK HYALOMMA CF. AEGYPTIUM

PAPERNA I.*, KREMER-MECABELL T.* & FINKELMAN S.*

INTRODUCTION

Ticks and mites have been shown to vector Hepatozoon of mammalian (Miller, 1908; Hoogstraal, 1961; Furman, 1966; Mathew et al., 1999) and avian hosts (Bennet et al., 1992); there is, however, only one report of reptile-host Hepatozoon sporogenic development in a tick (in Amblyoma dissimilae; Ball et al., 1969). Hemogregarinids of the genus Hemolivia, infecting toads (Bufo marinus), lizards (Tiliqua rugosa) and land tortoises (Testudo graeca) are tick-transmitted, and differ considerably in their course of development in their vector from species of Hepatozoon infecting reptiles transmitted via insects, notably mosquitoes. In the first, in the tick host, oocysts remain in the gut cells to yield a progeny of mobile sporokinetes, which re-enter the gut tissue to proceed sporogenesis (Petit et al., 1990; Smallridge & Paperna, 1997; Landau & Paperna, 1997; Smallridge & Paperna, 2000 a,b). In the latter, developing in dipteran insects (predominantly mosquitoes), oocysts escape into the hemocoel, sporocysts develop and remain to sporulate within the oocyst body (Lowichick et al., 1993). Hepatozoon of mammalian and avian hosts, undergo similar development into sporocyst loaded oocysts in their vector’s hemocoel (Bennet et al., 1992; Mathew et al., 1999). A few species of named Hepatozoon species have been reported to be transmitted via mites (Lewis & Wagner, 1964; Allison & Dessier, 1981), their potential affiliation, however, with the mite-transmitted hemogregarinid genus Karlyohus (Reichenow, 1921; Svanh, 1975) needs to be re-examined.

The starred lizard, Agama stellio, is widespread throughout the Middle East (Haas, 1951). Desser & Yeku-tiel (1986-1987) examined blood smears from starred lizards collected from different localities in Israel and Palestine and found two morphologically distinct types of undescribed hemogregarinids, stout and elongate. The elongate hemogregarine is an as yet undescribed specie of Hepatozoon (vide sp. A), which was transmitted experimentally via mosquitoes (Culex pipiens, Finkelman & Paperna, unpublished).

Summary:
Hepatozoon kisrae n. sp. was found infecting a starred lizard at a site in southeastern Samaria, Palestine. These lizards were also hosts to the ixodid tick Hyalomma cf. egyptium, which was demonstrated to be the vector of this hemogregarine. Hepatozoon and tick infections occurred in lizards within a very restricted locality; at a second site, nearby, ticks occurred without Hepatozoon infection. Micro- and macromeronts occurred mainly in the lungs, while cyst-like merogonic stages, mainly dizoic, occurred in the liver. Mature intraerythrocytic gametocytes were stout and encapsulated. Development from oocysts to sporocysts took place in the tick hemocoel, and was examined by transmission electron microscopy. Lizards were successfully infected when fed on sporocyst-infected ticks or viscera of infected lizards. Ticks become infected when fed on infected lizards; sporogony was complete when the ticks reached adult stage, over 40 days after initial attachment.

KEY WORDS: Hepatozoon kisrae n. sp., Agama stellio, Hyalomma cf. aegyptium, development, transmission, sporogony, ultrastructure.

Résumé: HEPATOZOOON KISRAE N. SP., PARASITE DU LÉZARD AGAMA STELLIO EST TRANSMIS PAR LA TIQUE HYALOMMA CF. AEGYPTIUM

Hepatozoon kisrae n. sp. a été découvert chez Agama stellio dans une localité du sud-est de la Samarie (Palestine). Ces lézards sont également les hôtes de Hyalomma cf. aegyptium. Il est démontré que cet Ixodidé est le vecteur de l’Hémogrégarine. Le cycle ne s’effectue que dans une zone précise : les Tiques d’une région voisine sont indemnes. Les micro et macromérontes se trouvent essentiellement dans les poumons, alors que les kystes (la plupart contenant deux cystozoïdes) siègent dans le foie. Les gamétocytes intraérythrocytaires mûrs, de forme trapue, sont encapulés. Le développement depuis l’oocyste jusqu’au sporocyte, qui a lieu dans l’hémocèle de la Tique, a été étudié en microscopie électronique. Les lézards s’inféctent par ingestion, soit de Tiques infectées de sporocystes, soit de viscéres de lézards infectés. Les Tiques s’inféctent par repas sur lézards infectés ; la sporogonie est achevée 40 jours après la fixation de la nymphal, lorsque le stade adulte est atteint.

MOTS CLE : Hepatozoon kisrae n. sp., Agama stellio, Hyalomma cf. aegyptium, développement, transmission, sporogonie, ultrastructure.
In this communication we describe the species forming the stout gametocytes, *Hepatozoon kisrae* n. sp., which infects the starred lizard at sites in southeastern Samaria, Palestine. These lizards are also the hosts to an ixodid tick *Hyalomma* cf. *aegyptium*, which is demonstrated to vector this hemogregarine.

**MATERIALS AND METHODS**

Starred lizards (*A. stellio*) (n = 27) were captured between September 23, 1999 to August 24, 2000 on the southeastern slopes of the Samarian mountains in Palestine (1978 edition map of Israel grids: 155-170 N/182-187E), at altitudes of 550 to 700 m above sea level, in a semiarid Mediterranean habitat (~ 350 mm annual rainfall) consisting of rocky areas and terrace-fenced olive groves. The collection sites were the following: (a) a stone-fenced olive grove, < 1,000 m² in area (“Kisra”, grid 166N/184E); (b) its adjoining grounds, at a perimeter of 500 m (“near Kisra”); (c) at roadsides along two km on Akaba-Kisra road; (d) a site situated 13 km down this road to the south (“Roman camp”); and e) two localities situated three and five km on the road branching eastwards from Kisra (sites c-e are listed as “elsewhere” in table I).

Blood smears were obtained by clipping the lizard’s toe tip. Ticks were opened by incision and their visceral contents were examined by direct light microscopy and from stained smears. Prepared blood films and smears were air-dried, fixed in absolute methyl alcohol and stained for one hour in Giemsa for microscopic examination. Engorged larvae and nymphs released by small incision, were examined directly and/or from air-dried, methanol-fixed, Giemsa-stained smears. Positive ticks were used either for an ultrastructural study, or to infect lizards via feeding.

Captive lizards were kept, each in a glass terrarium 50 x 30 x 30 cm in size under continuous illumination at room temperature of 25-31°C and were fed on mealworms supplemented by chopped chicken liver and meat. Experimental infection of lizards was carried out through their feeding on sporocysts dissected from naturally and laboratory infected ticks (*H. cf. aegyptium*); 2) by feeding on blood clots and viscera (mainly liver) of euthanized infected lizards. Together with the starred lizards, two geckoes (*Ptyodactylus basselquistii*) were also fed on viscera of infected ticks.

Infection-free lizards were obtained from habitats other than the enzootic location, and checked over two week period to verify the absence of infection. Visceral contents of the ticks: males, semi-engorged adult stages and nymphs released by small incision, were examined directly and/or from air-dried, methanol-fixed, Giemsa-stained smears. Positive ticks were used either for an ultrastructural study, or to infect lizards via feeding.

Engorged female ticks removed from captured lizards were kept in individual vials for oviposition, in slightly moistened chambers (~ 70-90 % RH) at ambient room temperature of 25-31°C. Laid eggs were incubated, and engorged larvae and nymphs were allowed to molt in vials under same conditions of temperature and humidity. Hungry larvae, nymphs and adults were stored in a 16°C incubator. Lizards, left free or caged in a net-box, were exposed to larvae or nymphs in containers with the rims of their tops aligned by sticky bands.

For transmission electron microscopic (TEM) examination, ticks were immersed in 2.5 % glutaraldehyde in cacodylate buffer (0.1 M, pH 7.4), tick abdomens was then sectioned at the anterior and posterior extremities, and either left that way or followed by progressive separation of the digestive tract from the chitinous envelope of the tick. The tick material left in the same fixative for 24 h at 4°C, was then rinsed in 0.1 M cacodylate buffer and postfixed in 1 % osmium tetroxide in the same buffer for one hour. After rinsing in the

<table>
<thead>
<tr>
<th>Localities</th>
<th>Kisra (site a)</th>
<th>Near Kisra (site b)</th>
<th>Elsewhere (sites c-e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no lizards examined</td>
<td>10</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>No. lizards infected with ticks</td>
<td>7</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>No. infected with <em>H. kisrae</em></td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. infected with <em>Hepatozoon</em> spp. A</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>No. lizards with <em>Hepatozoon</em>-infected ticks</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table I. – Summary of numbers of starred lizards examined from localities in Samaria, found infected by *Hepatozoon* spp. and found infested by *Hepatozoon*-infected and non-infected ticks.
buffer, the material was dehydrated in graded ethanol and embedded in Agar 100 medium (Agar Scientific, Ltd., UK). Thin sections, cut on a Reichert Ultracut microtome with a diamond knife were stained on grids with uranyl acetate and lead citrate, and examined with a Jeol 100CX TEM. Semithin sections cut on the same microtome were stained with toluidine blue for examination by light microscopy.

RESULTS

SPATIAL DISTRIBUTION OF HEPATOZOOON AND TICKS AMONG LIZARDS

All but one of the lizards (n = 10) caught in the olive grove in Kisra were infected with Hepatozoon kisrae n. sp.; of these, seven were infested with the tick Hyalomma cf. aegyptium (Table I). The only lizard found non-infected by H. kisrae was free from ticks. Ticks removed from the H. kisrae-infected lizards hosted either mature sporocysts or premature sporogony stages (Table II), with the exception of ticks removed from the low-infected lizard (No. 2, Table II), which were not infected. None of the lizards caught outside the olive grove, either nearby (n = 2), or elsewhere (n = 15) were infected with H. kisrae. Three of the latter lizards were infected by the mosquito-transmitted Hepatozoon sp. A. Ticks were found on one lizard from site b (a larva), on two in site c (a nymph and a male, both lizards were also infected with Hepatozoon sp. A), and on two lizards in site d (a male and two unengorged females); none were found hosting developing stages of Hepatozoon (Table II).

DESCRIPTION OF HEPATOZOOON KISRAE N. SP.

Hosts: Starred lizard, Agama stellio; the tick Hyalomma cf. aegyptium.

<table>
<thead>
<tr>
<th>Lizard marking</th>
<th>Date</th>
<th>Infected by H. kisrae</th>
<th>No. ticks present</th>
<th>No. examined</th>
<th>No. infected</th>
<th>With mature sporocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>September 23, 1999</td>
<td>+</td>
<td>0</td>
<td>1M, 1seN</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>September 23, 1999</td>
<td>±</td>
<td>1M, 2eN</td>
<td>1M, 1seN</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>September 23, 1999</td>
<td>++</td>
<td>1M, 1F, 2eN, 1eL</td>
<td>1M, 1F</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>6B</td>
<td>October 15, 1999</td>
<td>0</td>
<td>0</td>
<td>1M, 1F</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>8C</td>
<td>May 5, 2000</td>
<td>+++</td>
<td>3M, 1F, 3eL</td>
<td>1M, 1F</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>9C</td>
<td>May 5, 2000</td>
<td>+++</td>
<td>3M, 4F</td>
<td>1M, 3F</td>
<td>1M, 2F</td>
<td>1M</td>
</tr>
<tr>
<td>10C</td>
<td>May 5, 2000</td>
<td>++</td>
<td>0</td>
<td>1M, 1F</td>
<td>1M</td>
<td>1</td>
</tr>
<tr>
<td>11C</td>
<td>May 24, 2000</td>
<td>++</td>
<td>1M, 1F</td>
<td>1M, 1F</td>
<td>1M</td>
<td>1</td>
</tr>
<tr>
<td>2K</td>
<td>August 24, 2000</td>
<td>++</td>
<td>10seN</td>
<td>3seN, 1#M</td>
<td>1M</td>
<td>1M</td>
</tr>
<tr>
<td>3K</td>
<td>August 24, 2000</td>
<td>+</td>
<td>3seN, 1M</td>
<td>1M, 1#M, 1#F</td>
<td>1M, 1#M, 1#F</td>
<td>1M, 1#M, 1#F</td>
</tr>
</tbody>
</table>

Levels of parasitaemia: ± light (< 0.5 %), + low (0.5-3 %), ++ moderate (3-9 %), +++ high (> 9 %).

Table II. – Analysis of the state of infection of starred lizards and ticks collected in Kisra olive grove (site a); ticks: M-male, F-female, N-nymph, L-larva, e-engorged, se-semiengorged. * Immature ticks were allowed to molt before examination.
Fig. 1. – Merogonic stages of *Hepatozoon kisrae* n.sp. from lungs and liver of *Agama stellio* (× 1,300). A-G, stages in lungs: A. macromeronts in division (a) and divided micromeronts (i); B. Macromeronts after division; C. Macromeronts prior division; D. Post-division macromeront formation; E. Micromeronts prior division; F,G. Post division micromeront formations. H-L: Dizoic cyst-type meronts in the liver (L, merozoites show blue-staining crystalline-like bodies, marked with arrows).
Fig. 2. – Stages of *Hepatozoon kisrae* n. sp. from *Agama stellio* (× 1,300). A-D, from the liver: A. Four-zoite cyst-like meront; B. Meront dividing by polyendodyogeny; C. Polyzoic meront (macromeront); D. Undivided meront (arrow) and intraerythrocytic gametocytes (d). E-G: Gametocytes in the blood. E. Oval young (a) and premature with spirale nucleus (c); F. Premature with dense nucleus (b) and mature, encapsulated (d); G. Mature encapsulated; H. *Hepatozoon* sp. A. in blood of *A. stellio* from site e.
Table III. - Protocol of necropsies of 4 infected starred lizards: Exoerythrocytic *z*-zoites (cystozoites), Mi-micromeronts, Ma-macromeronts, Gam-gametocytes in blood. Parasite stages in the tissue: ± scarce, + a few, ++ prevalent, +++ numerous, ++++ very numerous.

<table>
<thead>
<tr>
<th>Lizard marking</th>
<th>Date infected</th>
<th>Onset of parasitaemia</th>
<th>Necropsy</th>
<th>Liver</th>
<th>Lungs</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>natural</td>
<td>(before 23.9)</td>
<td>after 167 days</td>
<td>z2 +</td>
<td>Mi &amp; Ma ++</td>
<td>Gam ++</td>
</tr>
<tr>
<td></td>
<td>Collected September 23, 1999</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>natural</td>
<td>(before 23.9)</td>
<td>after 84 days</td>
<td>z2 ++, z4.8</td>
<td>Mi &amp; Ma ++++</td>
<td>Gam ++</td>
</tr>
<tr>
<td></td>
<td>Collected September 23, 1999</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2B</td>
<td>November 7, 1999 by sporocysts</td>
<td>32 dpi</td>
<td>120 dpi</td>
<td>z2 ++++, z4.8</td>
<td>Mi &amp; Ma 16.52 +</td>
<td>?</td>
</tr>
<tr>
<td>7B</td>
<td>March 7, 1999 by lizard viscera</td>
<td>&lt; 37 dpi</td>
<td>37 dpi</td>
<td>z2+, z8 ±</td>
<td>?</td>
<td>Gam ++</td>
</tr>
</tbody>
</table>

Development in the tick
Syzgy was not detected. Oocysts, 67-74 x 47-54 μm (n = 4) in size, with expanded vacuolated cytoplasm, and a nucleus with a conspicuously large nucleolus, were found in the hemocoel, on the gut surface of engorged nymphs (Fig. 3A, B). Via segmentation (Fig. 3C), sporoblasts formed aggregates of sporocysts, 43-50 x 24-27 μm (n = 10) in size (Fig. 3D). Early differentiated sporocysts in engorged nymphs showed the already split crystalline bodies (Fig. 3E). Oocyst progeny consisted of over 100 sporocysts. The formed sporocysts remained aggregated within the oocyst, forming a ball-like structures (200-230 x 230 μm in size) in the hemocoel (Fig. 3F) visible to the naked eye as white balls. Mature oocysts with ripe sporocysts were found in unengorged adult male and female ticks and in nymphs, which failed to complete engorgement. Individual sporocysts, encased in a hardened wall, varied in size from 34 x 24 to 61 x 23 μm, and contained 16 to 35 sporozoites (Fig. 3G).

**ULTRASTRUCTURAL OBSERVATIONS ON SPOROCYST DEVELOPMENT**

Young sporocysts which had split from the sporoblast (Fig. 4A) gradually exhausted their stored amylopectin granules as well as their lipid vacuoles (Fig. 4B). Small granular aggregates – the anlagen of crystalloid bodies, enclosed within rough endoplasmic reticulum (ER), grew to large inclusions of granular matrices (Fig. 4B, C), which became consolidated into arrays of crystalloids (Fig. 4D, E). The ER network also incorporated numerous tubular mitochondria (Fig. 4B). Small electron-dense bodies remained in the sporocyst cytoplasm to late stage of differentiation (Fig. 4A, B, D). The rough ER, which accompanied the forming crystalloid bodies, disappeared when the crystalloid arrays became consolidated (Fig. 4D, E). The single nucleus of the formed sporocysts divided. All formed nuclei had a conspicuous, often aggregated nucleolus (Fig. 4D, E). At this stage encapsulation started, the future hard wall consolidated above the plasmalemma, and became superimposed by a fine veil (Fig. 4F). Increased hardening of the sporocyst wall accelerated its resistance to fixation and impregnation, which eventually precluded further ultrastructural analysis.

**EXPERIMENTAL INFECTIONS**

From ticks to lizards
Six starred lizards (all adults), from sites other than Kisra, verified to be non-infected, were fed on sporocysts-infected *H. cf. aegyptium* ticks (Table IV). Five were fed, each on a tick removed from naturally infected lizard and one (7K) on a tick experimentally engorged on an infected lizard. The latter lizard developed the same infection schedule as the ones fed on naturally infected ticks. All but one developed parasitaemia not later than 32 to 40 days post-infection (p.i), in the latter (1C), light infection with young gametocytes was detected only 90 days p.i. In two lizards blood already contained mature gametocytes by day...
Fig. 3. - Developmental stages of Hepatozoon kisrae n. sp. in the tick Hyalomma cf. aegyptium. A-E, toluidine-stained semithin sections; F,G, unfixed, live: A,B. Oocysts, × 1,156 and × 462; C. Oocyst (o) dividing into sporocysts (s) × 500; D. Sporocysts × 887; E. Sporocysts with divided crystalline bodies × 939; F. Oocyst ball filled with ripe sporocysts × 324; G. Single sporocysts filled with sporozoites × 1,968.
Fig. 4. – Electron microscopic images of sporocysts from infected ticks. A. Newly formed sporocyst filled with amylopectin granules, showing anlagen of crystalloid bodies enclosed in rough ER (pr) mitochondria (m) and electron-dense bodies (d); × 7,150. B. Sporocyst with ER-aligned inclusion of pre-crystalloid granular bodies (r), showing also mitochondria (m) and lipid vacuoles (L); × 6,400. C. Sector of divided sporocysts showing a nucleus (n) with scattered nucleolus (ns), and ER (er) aligning the pre-crystalloid granular body (r); × 10,344. D,E. Sporocysts with several nuclei (n), arrayed crystalloid bodies (r), amylopectin granules (a) and electron-dense bodies (d); × 6000 and × 6,460. F. Walling process of a sporocyst, the forming wall is invested by a veil (arrowhead); In addition to the formed crystalloid body (r) a crystalloid anlage (pr) enclosed in ER still occurs; a, amylopectin granules; × 15,300.
37 p.i., in further two by day 40 p.i. and in one by
day 60 p.i.
The two geckoes fed simultaneously with 1K and 4K
starred lizards on sporocyst-infected tick viscera, failed
to develop infection by day 40 p.i.

From starred lizard to starred lizard
Three lizards (two juveniles and one adult from out­
side Samaria) were fed on blood and liver obtained
from a euthanized infected lizard (lizard 1, Table II).
Parasitaemia, including of mature encapsulated gam­
etocytes was detected by day 37 in the adult and 47
days p.i. in both juvenile lizards.

From lizards to tick
Two H. kisrae-infected lizards, and three non infected
ones were exposed to H. cf. aegyptium larvae, en­
larged larvae were obtained after 11-13 days post-ex­
sure, some of the larvae proceeded without descend­
ing to the nymph stage and dropped off as engorged
nymphs 30 to 38 days later. None of these, engorged
larvae or hungry nymphs, recovered up to 40 days
post-attachment were found infected. Infection was,
however, found in spontaneously detached semi­
engorged nymphs (n = 6) who failed to molt, ex­
amined 41 days post initial attachment. Infection was
already comprised of mature sporocysts with sporo­
zites. Adult ticks developing from molting engorged
nymphs were found infected by non-sporulated
oocysts, as well as oocysts containing non-differenti­
ted and mature sporocysts. These ticks were 41 to 77
days after initial exposure.

All 10 dissected adult ticks and three engorged nymphs
recovered after a feeding schedule on three Hepato­
zoon sp. A.-infected lizards examined either 42 to 77
days (adults) or 20 to 42 days (engorged nymphs) after
initial attachment were negative. One engorged nymph
examined 22 days post-attachment contained Hepato­
zoon sp. A. gametocytes.

Transovarian transmission
Infection was not traced in any of the progeny grown
from ovigerous female removed from infected lizard
from Kisra. Examined ticks were nymphs (3) and
adults (10) engorged after feeding on three non­
infecteted lizards.

**DISCUSSION**

Agama stellio, the starred lizard is abundant and
widely distributed in the Near East and has
become a peridomestic inhabitant (Haas, 1951).
On the otherhand the spatial distribution of both ticks
and H. kisrae infection in starred lizards appears to be
very patchy, the present locality was restricted to a
stone fence-enclosed area of less than 1,000 m2. Desser
& Yekutiel (1986-1987) reported Hepatozoon infection
by stout gametocytes, apparently conspecific with
H. kisrae, in starred lizards from three localities: two
in the same arid geographical subregion (in south-east
Samaria), and one in the forested Mediterranean zone
in west Jerusalem, with ~ 800 mm annual rainfall.
Hyalomma cf. aegyptium were never been found on the
previously investigated starred lizards (Ostrovska &
Paperna, 1987; Bristowetzki & Paperna, 1990). As was
found in our study, not all patches of tick infection
necessarily generate active H. kisrae transmission. The
species of Hepatozoon co-habiting starred lizards are
readily distinguishable, and appears to demonstrate a
strict specificity to their respective vector hosts, H. kis­
rae, with the stout gametocytes, to H. cf. aegyptium
and Hepatozoon sp. A. With the elongate gametocytes,
to mosquitoes (Culex pipiens, Finkelman & Paperna,
unpublished). Transmission by mosquitoes could favor
a more continuous pattern of distribution for Hepato­
zoon sp. A. The spatial distribution of this apparently
more abundant species, however, is also patchy,
though over seemingly a wider range of habitats (Fink­
elman & Paperna, unpublished).

H. kisrae appears to have narrow host specificity, pos­
sibly only A. stellio, infection failed to established itself
in geckoes (P. basselquistii) fed on infected ticks.
The vector tick's identity remains inconclusive. The
ticks found on the lizards, by all morphological criteria
outlined by Hoogstraal (1956), are indistinguishable
from H. aegyptium, which has been found feeding pre­
dominantly on land tortoises (Testudo graeca) and
vectors Hemolivia mauritiana (Michel, 1973; Landau
& Paperna, 1997). Although Hoogstraal (1956) notes
that rarely the tick was found feeding on A. stellio, in
our experiments (unpublished), larvae, nymphs and
adults reared from the ticks recovered from the lizards, refused to attach to tortoises.

Ball et al. (1969) found sporozoite stages in the tick *Amblyoma dissimile* as well as in mosquitoes (*Culex tarsalis, Aedes togoito*), when fed on same Hepatozoon fusifex-infected *Boa constrictor*. The parasites, which developed in the tick, as authors also admit, however, do not necessarily have to be conspecific with the ones found in the mosquitoes. These mosquitoes failed to acquire infection when fed on snakes infected via *A. dissimile*.

Apparently, all true members of the genus Hepatozoon, as well as species of *Hemolivia* have a dichotomous course of merogony, one of active large-progeny merogonies to sustain the subsequent gamogonous generation in the blood and the other, yielding persistent cyst-like stages. These cyst-enclosed stages were termed cystozoites (Landau et al., 1972). They were transmitted when ingested as prey by a subsequent vertebrate host (Landau et al., 1972; Smith & Dessir, 1998). In our experiments, active merogonic stages could not be separated from the cyst-like stages in the viscera used to infect lizards via feeding.

In *H. kisrae*, active merogonies developed in endothelial cells in the lungs and to a lesser extent in the liver. Cystozoites occur only in the liver, mostly in macrophages in MMC. In *A. stellio* experimentally infected by *Hemolivia mariae*, a few cystozoites also developed in the lungs, in formed MMC (Smallridge & Paperna, unpublished).

The large-progeny merogonies are formed by polyendodyogenous merogony, and generations of 4 to 16 large meronts are followed by one or more eight to 32 progeny-generations of micromeronts, which escape to the blood to develop into gametocytes. The dicrozoic cystozoites have been seen to form by endodyogeny (Landau et al., 1972), as also seen in this study: cystic stage-four and eight zoites are generated by successive endodyogenies.

Development of the presently described species, although taking place in ticks, occurs similarly to that seen in mosquitoes, i.e. within the oocyst located in the tissue lining the haemocoeil (Bashtar et al., 1984; Lowi-chik et al., 1993; Smith & Dessir, 1997). In *Hemolivia* developing in ticks the sporocysts released (as sporokinetes) from the oocyst remain located inside parasitophorous vacuoles within the tick gut cells (Smallridge & Paperna, 2000b).

In ultrastructurally examined *H. kisrae* sporocysts, the crystallloid material initially assembled within pockets of ER, by the same route as seen in species developing in mosquitoes (*H. domerguei, Vivier et al., 1972; H. aegyptii, Bashtar et al., 1984*), and similarly led to the formation of large crystalloid arrays which are split between the ultimately formed sporozoites. Although the developmental process in *Hemolivia* also involves formation and split of crystalloid substance between the offspring, it seems to proceed differently. The extensive ER, which accompanies the forming crystallloid bodies in *Hepatozoon*, is lacking in *Hemolivia* (or disappears at an earlier stage of differentiation). In *Hemolivia* the crystallloid substance is first split between sporokinetes (Smallridge & Paperna, 2000a), and only then between the sporozoites (Paperna & Smallridge, 2000b).

---

**REFERENCES**


Ball G.H., Chao J. & Telford S.R. The life history of Hepatozoon rarefaciens (Sambon and Seligmann, 1907) from Drymarchon corais (Colubridae) and its experimental transfer to Constrictor constrictor (Boidae). *Journal of Parasitology* 1967 53, 897-909.

Ball G.H., Chao J. & Telford S.R. Hepatozoon fusifex sp. n. a hemogregarine from Boa constrictor producing marked morphological changes in infected erythrocytes. *Journal of Parasitology* 1969, 55, 800-813.


Haas G. On the present state of our knowledge of the herpetofauna of Palestine. *Bulletin of the Research Council of Israel*, 1951, 1, No. 3 (August).


---


MILLER W.W. Hepatozoon perniciosum n.g. n.sp., a hemogregarine pathogenic for white rats; with a brief description of the sexual cycle in the intermediate host, a mite (Laelaps echidnus Belles). Bulletin of the Hygiene Laboratory of Washington, 1908, 46, 51-123.


SMALLRIDGE C. & PAPERNA I. Ultrastructure of Hemolivia mariae gamonts in the blood of the lizard Tiliqua rugosa and their development to oocyst stage in the tick Amblyomma limbatum. Parasitology Research, 2000b, 86, 563-569.
