HEPATOZOON KISRAE N. SP. INFECTING THE LIZARD *AGAMA STELLIO* IS TRANSMITTED BY THE TICK *HYALOMMA* CF. *AEGYPTIUM*

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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. *aegyptium*, 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 : *HEPATOZOON 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 macrométrontes se trouvent essentiellement dans les poumons, alors que les kystes (la plupart contenant deux cystozoïtes) siègent dans le foie. Les gamétocytes intraérythrocytaires mûrs, de forme trapue, sont encapsulés. Le développement depuis l'oocyste jusqu'au sporocyste, qui a lieu dans l'hémocèle de la Tique, a été étudié en microscopie électronique. Les Lézards s'infectent par ingestion, soit de Tiques infectées de sporocystes, soit de viscères de Lézards infectés. Les Tiques s'infectent par repas sur Lézards infectés ; la sporogonie est achevée 40 jours après la fixation de la nymphe, lorque le stade adulte est atteint.*

MOTS CLÉS : Hepatozoon kisrae *n. sp.*, Agama stellio, Hyalomma *cf.* aegyptium, *développement, transmission, sporogonie, ultrastructure.*

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* sporogonic development in a tick (in *Amblyoma dissimilae*, Ball *et al.*, 1969). Hemogregarines 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, unergo 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 & Desser, 1981), their potential affiliation, however, with the mite-transmitted hemogregarinid genus *Karyolysus* (Reichenow, 1921; Svahn, 1975) needs to be re-examined.

The starred lizard, *Agama stellio*, is widespread throughout the Middle East (Haas, 1951). Desser & Yekutiel (1986-1987) examined blood smears from starred lizards collected from different localities in Israel and Palestine and found two morphologically distinct types of undescribed hemogregarines, 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).

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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 m^2 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 Akraba-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 (10 % in phosphate buffer pH 7.4). Levels of parasitaemia defined as: ± light (< 0.5 %), + low (0.5-3 %), ++ moderate (3-9 %), +++ high (> 9 %) were determined from counted 50 fields at × 1,000 magnification (~ 400 erythrocytes per field).

From moribund, euthanized and freshly dead lizards, tissue samples of the liver, lungs and sometimes spleen were fixed for histological examination in 10 % neutral-buffered formalin. After dehydration in graded ethanols, the tissues were embedded in glycol-methacrylate medium (GMA of Agar Comp., UK). Sections, 3 µm thick, were cut with a glass knife on a Sorval JB4 microtome and stained with either Meyer's haemalum-eosin or, after a 20 min incubation in Bouin's solution and a rinse with water and 70 % ethanol, with 10 % Giemsa, in phosphate buffer pH 7.4, for one hour.

Captive lizards were kept, each in a glass terrarium 50 × 30 × 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 1) 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 hasselquistii*) 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 abdomen 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

Localities	**Kisra** (site a)	**Near Kisra** (site b)	**Elsewhere** (sites c-e)
Total no lizards examined	10	2	15
No. lizards infested with ticks	7	1	4
No. infected with *H. kisrae*	9	0	0
No. infected with *Hepatozoon* sp. A	0	0	3
No. lizards with *Hepatozoon*-infected ticks	6	0	0

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 ethanols 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 *HEPATOZOON* 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 *Hyaloma* 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 *HEPATOZOON KISRAE* N. SP.

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

Type locality: Olive grove at Kisra (grids 182E /162N), southeast Samaria, Palestine.

Etymology: Named after the type-host locality.

Stages in the starred lizard

Merogonic stages dividing by polyendodyogeny (Fig. 1A), seen in the lungs, were encapsulated (29-39 × 15-22 µm in size, n = 7) inside endothelial cells, comprised of either macromeronts dividing into 4 to 16, 11-16 × 3-4 µm merozoites (Fig. 1 A-D), or micromeronts dividing to 16 to 32, 8-11 × 1.5-2 µm merozoites (Fig. 1, A, E-G).

In the liver occurred the cyst-type meronts or cystozoites, with two ("dizoic", 19-24 × 12-17 µm in size, n = 7, Fig. 1H-L), four (20-28 × 10-15 µm in size, n = 7, Fig. 2A) and exceptionally eight zoites. These cysts were lodged within melanomacrophage centers (MMC), evidently inside macrophages. The zoite (12-15 × 2-5 µm in size, n = 12) couples were apparently formed by endodyogeny. They were enclosed in a meront residuum (the cystic body) filled with vacuolar matrix (visible only in histology). Some dizoites contained a blue-staining inclusion, which might be a crystalline body (Fig. 1I, L). Exceptionally large zoites, 22.2 × 3.8 µm, showed tapering, deep staining, anterior end (not shown).

The liver also contained a few merogonic stages, merogonic (polyendodyogenic) division inside an endothelial cell is shown in Figure 2B; macro and micromerogonic stages (both 23-29 × 15-18 µm in size) contained up to 16 (Fig. 2C) and 32 zoites, respectively. Liver tissue also contained free, single, un-divided meronts (Fig. 2D), reaching a size of 16-17 × 4-7 µm (n = 2).

Four lizards, two naturally infected, one infected by ingestion of a tick and one by infected lizard viscera (1), kept for 37 to 167 days, when necropsied, all demonstrated the presence of cyst-type meronts in the liver. In the two lizards also examined for lung infec-

Lizard marking	Date	Infected by *H. kisrae*	No. ticks present	No. examined	No. infected	With mature sporoöcysts
1	September 23, 1999	+	0			
2	September 23, 1999	±	1M, 2seN	1M, 1seN	0	
3	September 23, 1999	++	1M, 1F, 2seN, 1eL	1M, 1F	2	2
6B	October 15, 1999	0	0			
8C	May 5, 2000	+++	3M, 1F, 3eL	1M, 1F	2	2
9C	May 5, 2000	+++	3M, 4F	1M, 3F	1M, 2F	1M
10C	May 5, 2000	++	0			
11C	May 24, 2000	++	1M, 1F	1M, 1F	1M	1
2K	August 24, 2000	++	10seN	3seN, 1≠M	3eN, 1≠M	1≠M
3K	August 24, 2000	+	3seN, 1M	1M, 1≠M, 1≠F	1M, 1≠M, 1≠F	1M, 1≠M, 1≠F

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.

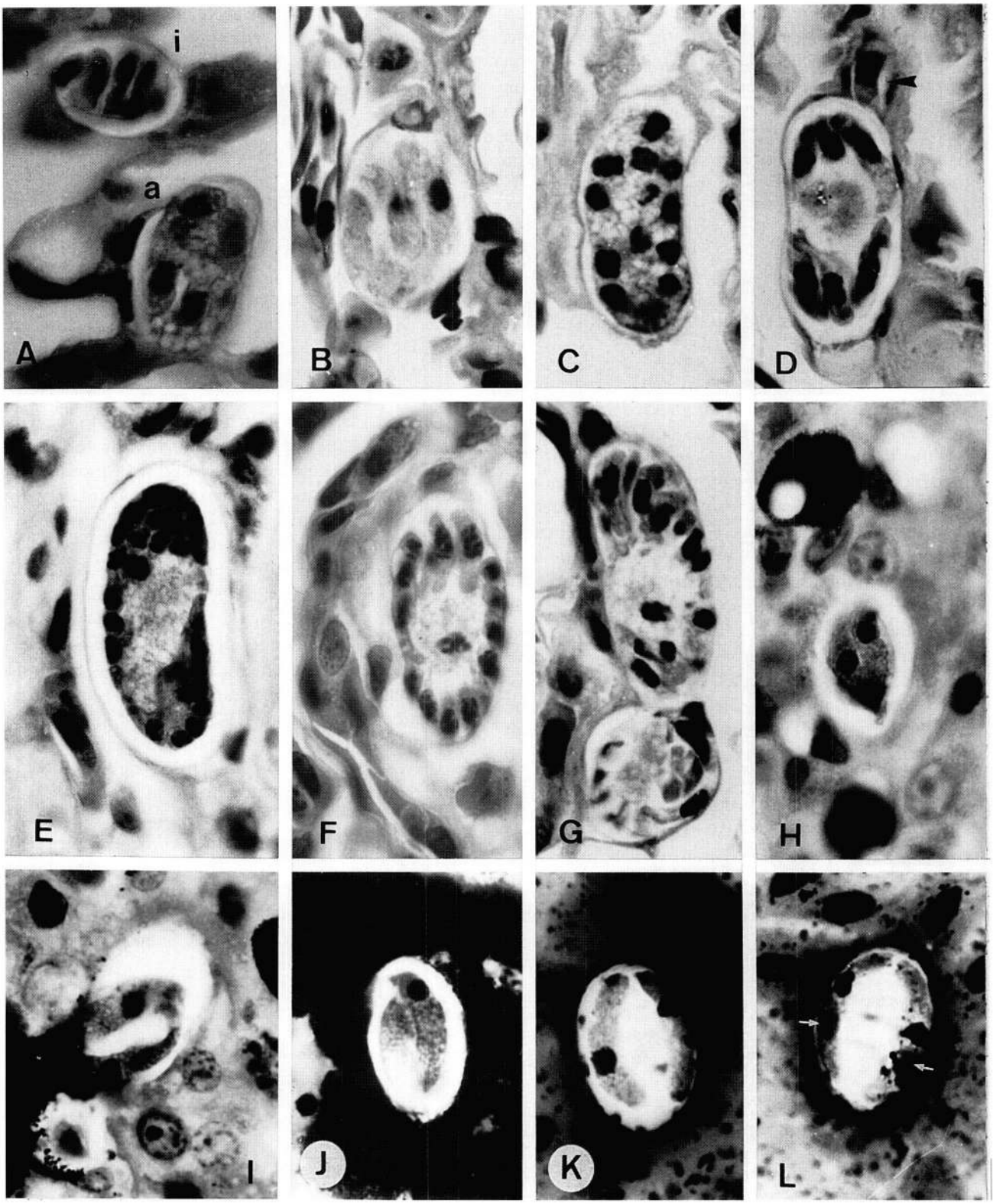

Fig.1. – Merogonic stages of *Hepatozoon kisrae* n.sp. from lungs and liver of *Agama stellio* (× 1,300). A-G, stages in lungs: A. macromeronts in division (a) and divided micromeronts (i); B. Macromeronts after division; C. Macromeronts prior division; D. Post-division macromeront formation; E. Micromeronts 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).

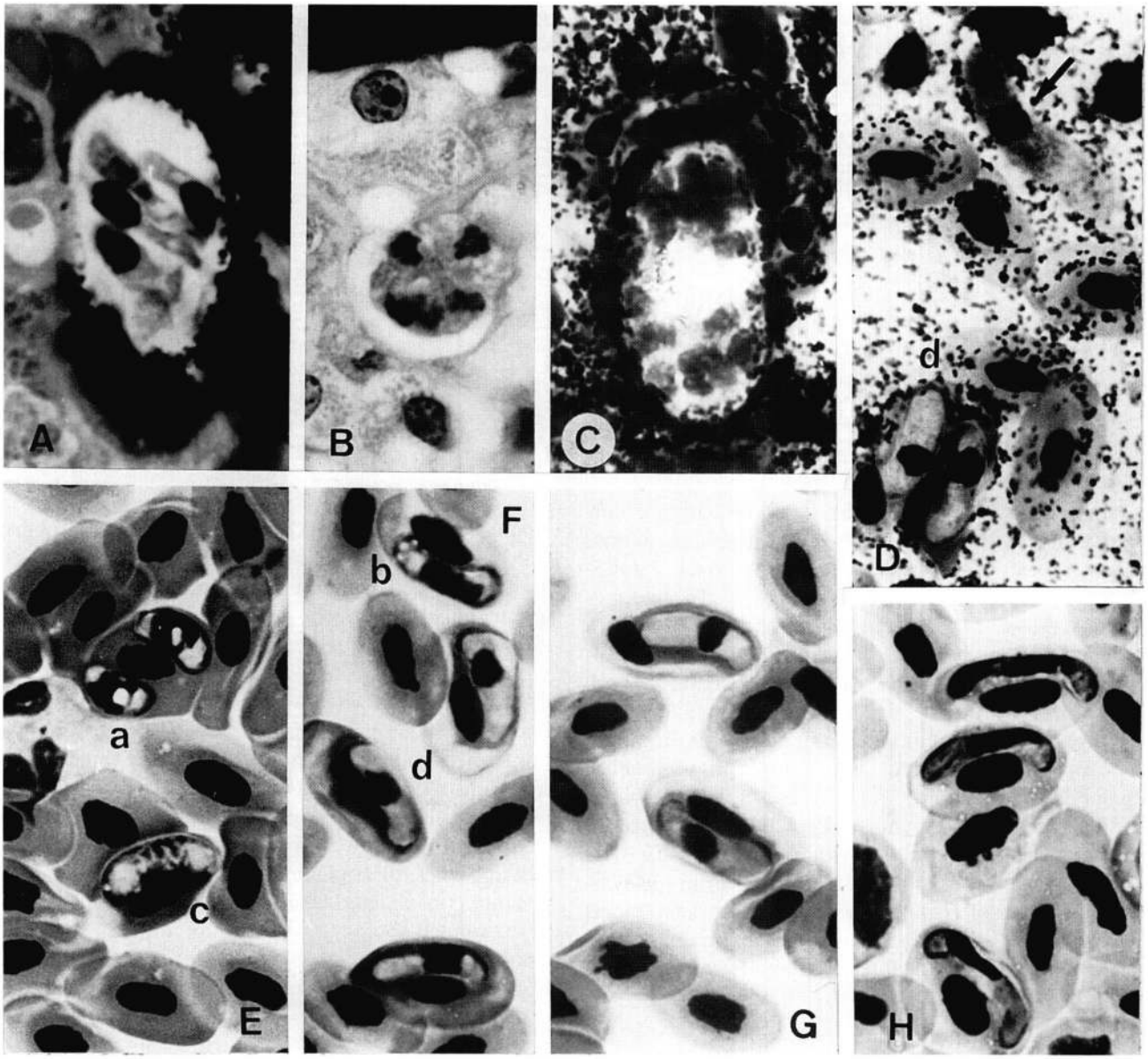

Fig. 2. – Stages of *Hepatozoon kisrae* n. sp. from *Agama stellio* (× 1,300). A-D, from the liver: A. Four-zoite cyst-like meront; B. Meront dividing by polyendodyogeny; C. Polyzoic meront (macromeront); D. Undivided meront (arrow) and intraerythrocytic gametocytes (d). E-G: Gametocytes in the blood. 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.

Lizard marking	Date infected	Onset of parasitaemia	Necropsy	Liver	Lungs	Blood
1	natural Collected September 23, 1999	(before 23.9)	after 167 days	z2 +	Mi & Ma ++	Gam ++
3	natural Collected September 23, 1999	(before 23.9)	after 84 days	z2 ++, z4,8	Mi & Ma ++++	Gam ++
2B	November 7, 1999 by sporocysts	32 dpi	120 dpi	z2 +++, z4, 8, Mi & Ma 16,32 +	?	Gam ++
7B	March 7, 1999 by lizard viscera	< 37 dpi	37 dpi	z2+, z8 ±	?	Gam ++

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.

tion, macro- and micromeront infection coincided with the liver infection (Table III).

Gametocytes at several stages of differentiation occurred in the erythrocytes. The earliest ones were oblong, 6-8 × 4-5 µm (n = 4), and subsequently oval, 7-9 × 5-6 µm, with a large central nucleus (Fig. 2E). Intermediate stages (7-11 × 5-7 µm, n = 4) had a nucleus with spirally scattered chromatin (Fig. 2E, F). The presumably fully mature stages were stout, banana-shaped, 12-15 × 2-5.5 µm in size (n = 9), with a centrally located 4 to 5.5 µm wide multilobed or homogeneously packed nucleus; the cytoplasm was either stained homogeneously pale, or had a variable quantity of vacuoles and granules (Fig. 2F, G). Fully mature gametocytes were encased within a hard capsule. The gametocytes were readily differentiated from the sympatric species of *Hepatozoon* found in *A. stellio* blood (*Hepatozoon* sp. A, Finkelman & Paperna, unpublished), the latter being elongate, with inward-bent ends (Fig. 2H).

Development in the tick

Syzygy was not detected. Oocysts, 67-74 × 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 × 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 × 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 × 24 to 61 × 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 resistence 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 parasitemia 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

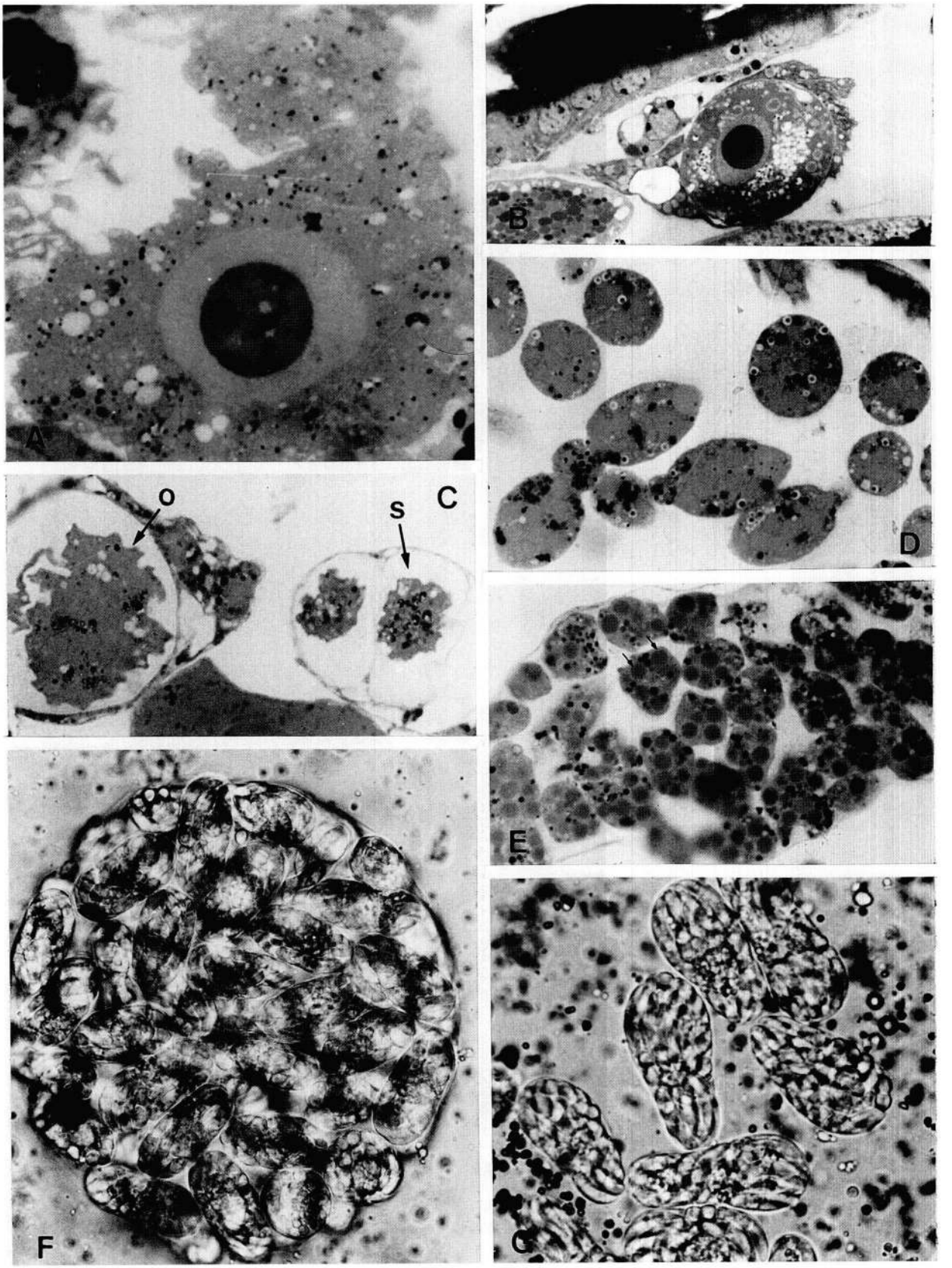

Fig. 3. – Developmental stages of *Hepatozoon kisrae* n. sp. in the tick *Hyalomma* cf. *aegyptium*. A-E, toluidine-stained semithin sections; F,G, unfixed, live: A,B. Oocysts, × 1,156 and × 462; C. Oocyst (o) dividing into sporocysts (s) × 500; D. Sporocysts × 887; E. Sporocysts with divided crystalline bodies × 939; F. Oocyst ball filled with ripe sporocysts × 324; G. Single sporocysts filled with sporozoites × 1,968.

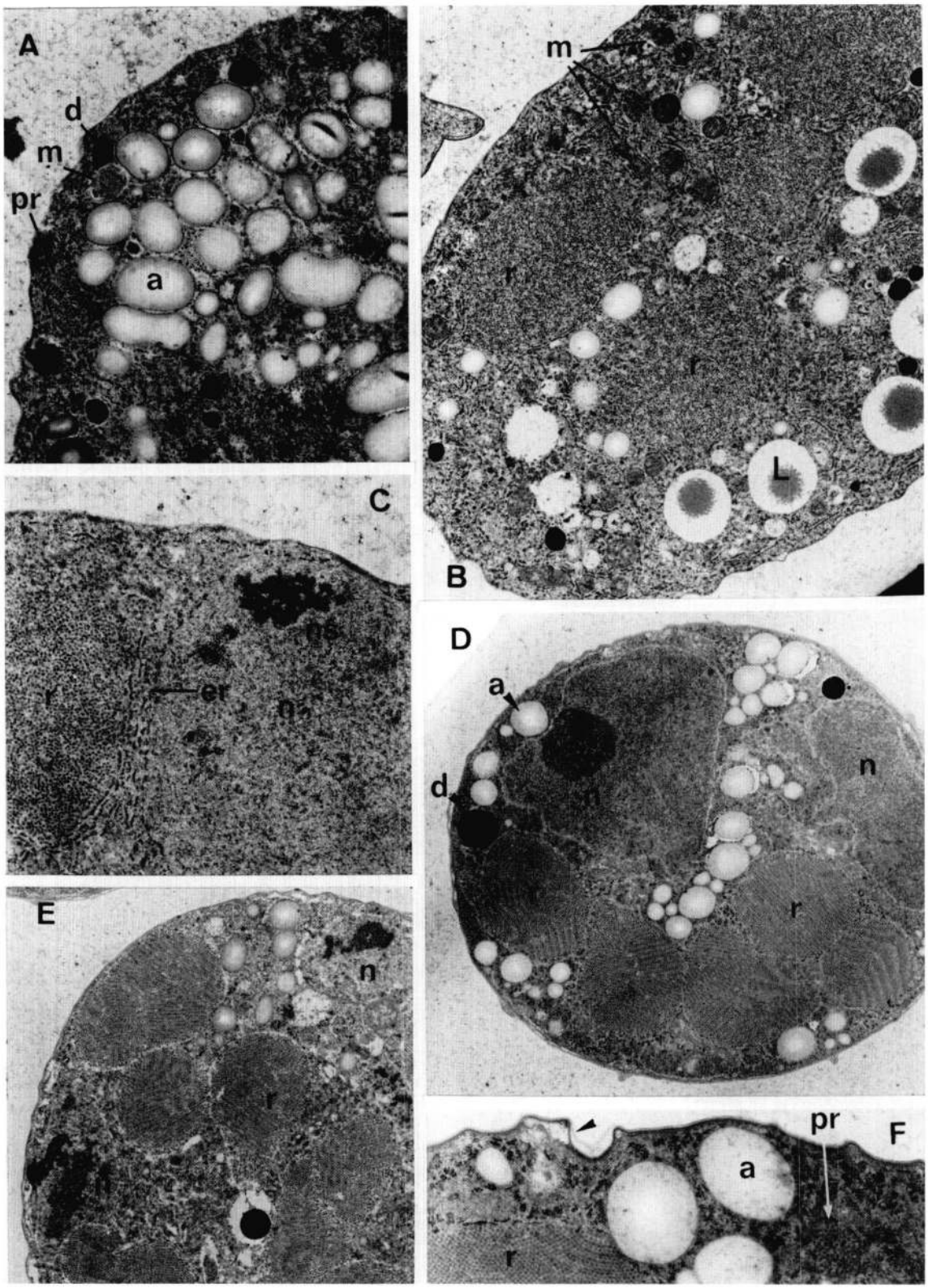

Fig. 4. – Electron microscopic images of sporocysts from infected ticks. A. Newly formed sporocyst filled with amylopectin granules, showing anlagen of crystalloid bodies enclosed in rough ER (pr) mitochondria (m) and electron-dense bodies (d); × 7,150. B. Sporocyst with ER-aligned inclusion of pre-crystalloid 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.

Lizard marking	Origin	Date	Date fed on sporocysts	Dpi to first seen infection	Dpi to mature gametocytes
2B	site d	October 15, 1999	November 7, 1999	32	60
1C	site c	May 5, 2000	May 15, 2000	93	~
1K	site b	August 24, 2000	October 17, 2000		40
4K	site b	August 24, 2000	October 26, 2000		40
5K	site e	August 24, 2000	September 3, 2000		37
7K	site d	August 24, 2000	September 3, 2000	< 37	37 (died)

Table IV. – Experimental infection of starred lizards by feeding on *H. kisrae* sporocysts from ticks.

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 outside Samaria) were fed on blood and liver obtained from a euthanized infected lizard (lizard 1, Table II). Parasitaemia, including of mature encapsulated gametocytes 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, engorged larvae were obtained after 11-13 days post-exposure, some of the larvae proceeded without descending 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, examined 41 days post initial attachment. Infection was already comprised of mature sporocysts with sporozoites. Adult ticks developing from molting engorged nymphs were found infected by non-sporulated oocysts, as well as oocysts containing non-differentiated 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 *Hepatozoon* 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 *Hepatozoon* 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-infected 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 m^2. 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. kisrae*, 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 *Hepatozoon* sp. A. The spatial distribution of this apparently more abundant species, however, is also patchy, though over seemingly a wider range of habitats (Finkelman & Paperna, unpublished).

H. kisrae appears to have narrow host specificity, possibly only *A. stellio*, infection failed to established itself in geckoes (*P. hasselquistii*) 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 predominantly on land tortoises (*Testudo graeca*) and vectors *Hemolivia mauritanica* (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 persistant 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 & Desser, 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 dizoic 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 moquitoes, i.e. within the oocyst located in the tissue lining the haemocoel (Bashtar *et al.*, 1984; Lowichik *et al.*, 1993; Smith & Desser, 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 crystalloid 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 crystalloid bodies in *Hepatozoon*, is lacking in *Hemolivia* (or disappears at an earlier stage of differentiation). In *Hemolivia* the crystalloid substance is first split between sporokinetes (Smallridge & Paperna, 2000a), and only then between the sporozoites (Paperna & Smallridge, 2000b).

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