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THE LIFE CYCLE OF *ISOSPORA ELMAHALENSIS* IN THE WHITE-CHEEKED BULBUL (*PYCNONOTUS LEUCOGENYS*)

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M. Abdulla Amoudi (1990) The life cycle of *Isospora elmahalensis* in the white-cheeked bulbul (*Pycnonotus leucogenys*). *Bull. Inst. Zool., Academia Sinica* **29**(4): 265-272. The life cycle of *Isospora elmahalensis* from *Pycnonotus leucogenys* is described from experimental infections. There were two asexual generations, both of which developed below or to the side of the nucleus of the host cell or to the side of the nuclei of the host cells. Mature 1st generation schizonts, seen 60 h after inoculation, were 12.5-14.5×7-9 μm (N=14). Mature 2nd generation schizonts were first seen 80 h after inoculation, and were 12.5-14×7-8.5 μm (N=13). Mature macrogametocytes with plastic granules were presented 100 h after inoculation, and were 10.5-12.5×9-11 μm (N=18). Mature microgametocytes were also found and were 12-14.5×10-11 μm (N=15). The mean prepatent period was 4.5 days and the patent period was from 5 to 6 days. The peak oocyst output occurred on the 3rd day of the patent period. Five fledgling *Isospora* free bulbuls fed on sporulated oocysts and started discharging oocysts on day 5 between 15:00 h and 21:00 h.

Key words: *Isospora elmahalensis*, *Pycnonotus leucogenys*, Schizonts, Residual cytoplasm, Plastic granules.

Only two species of the genus *Pycnonotus* are found in Saudi Arabia: *Pycnonotus leucogenys* and *P. xanthopygos* (Silsby, 1980). The former one is restricted mainly in the Eastern provinces and some parts of the Southwestern provinces of Saudi Arabia. This species is threatened with extinction because of habitat destruction which may be a result of the colossal petrochemical industry scheme, the construction already having been completed in Al Jubail on the Arabian Gulf; it is certainly going to have immediate and farreaching impacts on the fauna of the Eastern provinces, in addition to causing diseases that have already led to a decline in the numbers of wild species. The present efforts in conservation have been established through the initiative of the National Commission for Wildlife Conservation and Development (NCWCD). Coccidiosis is common in wildlife, especially in birds; but little is known about its biology. However many species of *Isospora* have been described from passerines. Levine (1982) listed 60 species of *Isospora* in such birds, and there is no doubt many more have yet to be discovered. The only species of *Isospora* described from Pycnotidae are *I. pycnonotus* and *I. pycnonoti*

(Bhatia *et al.*, 1973). The endogenous stages in the life cycle of only a few species of *Isospora* have been described. In Pycnonotidae only those of *I. pycnonotus* of *P. jocosus* have been described (Mandal and Chakravarty, 1964). My study describes the endogenous development of *I. elmahalensis* in the white-cheeked bulbul (*Pycnonotus leucogenys*). This study has performed a valuable service to the domains of animal health and it shall encourage others in dealing with problems of wildlife management in the Kingdom.

MATERIALS AND METHODS

Selection and Management

Twenty birds (11 males and 9 females) were purchased from local dealers in June of 1988, and were maintained in the laboratory for 10 days to ensure that they were free of coccidia as judged by fecal examination. The birds weighed from 29–40 gm and were held separately in cages measuring 45 cm×35 cm×50 cm. Non-absorbant paper was placed underneath for collecting feces. Birds was kept at 25°C and supplied with food (date palm) and water once a day.

Preparation of Inoculum

A culture of oocysts of *Isospora elmahalensis* (Amoudi, 1987) was established in our laboratory using the method described by Vetterling (1965). Each bird was given 15,000 sporulated oocysts of *I. elmahalensis per os* with the aid of a syringe fitted with plastic tubing, 1.19 mm outer diameter, *via* an 18 gauge needle.

Histological Preparation and Examination

Fifteen birds were killed and necropsied at 20 h intervals (chosen to give greater precision than the usual 24 h intervals). Tissues were collected from the duodenum, jejunum, ileum and large intestine and fixed in Zenker's solution. The tissues were processed by routine histologic methods and stained with Ehrlich's haematoxylin and eosin Y and examined with a Zeiss Universal Photomicroscope III equipped with a 100× objective lense. Sections were photographed with Panatomic X 35 mm film. Measurements were made using a calibrated ocular micrometer. Size ranges were followed by the number of stages examined (N) in parenthesis.

The five birds remaining were also caged separately and used in an experiment designed to establish whether there is diurnal periodicity of oocyst production. Feces were collected from each bird at 3 to 6 hour intervals and bulked into 24 hour samples between days 5 and 10 post inoculation. The fecal samples were weighed, homogenized, and the number of oocysts per gram were determined (Amoudi, 1988).

RESULTS

All of the intracellular stages were located in all parts of the small intestine. Endogenous stages were found below or to the side of the nuclei of the epithelial cells. Asexual stages developing from sporozoites were located beside the host cell nuclei which were slightly displaced by the parasite. Trophozoites and schizonts were in the crypts or in the middle of the villi. Sexual stages developed along the entire length of the villi, but mostly at the tips of the villi. Most of them were located inferior to the host cell nuclei between the nuclei and the basement of the cell and the nuclei were slightly indented adjacent to the parasite.

Schizogony

Two asexual generations developed. Trophozoites with refractile globules (Fig. 1) and binucleated schizonts with

Abbreviations used in Figs. 1-13; HN, host cell nucleus; N, nucleus; Nu, nucleolus; PV, parasitophorous vacuole; PG, plastic granules; RG, refractile globule; RC, residual cytoplasm.

Fig. 1. Trophozoite of *I. elmahalensis* with small nucleus; note relatively large refractile globule (arrow).

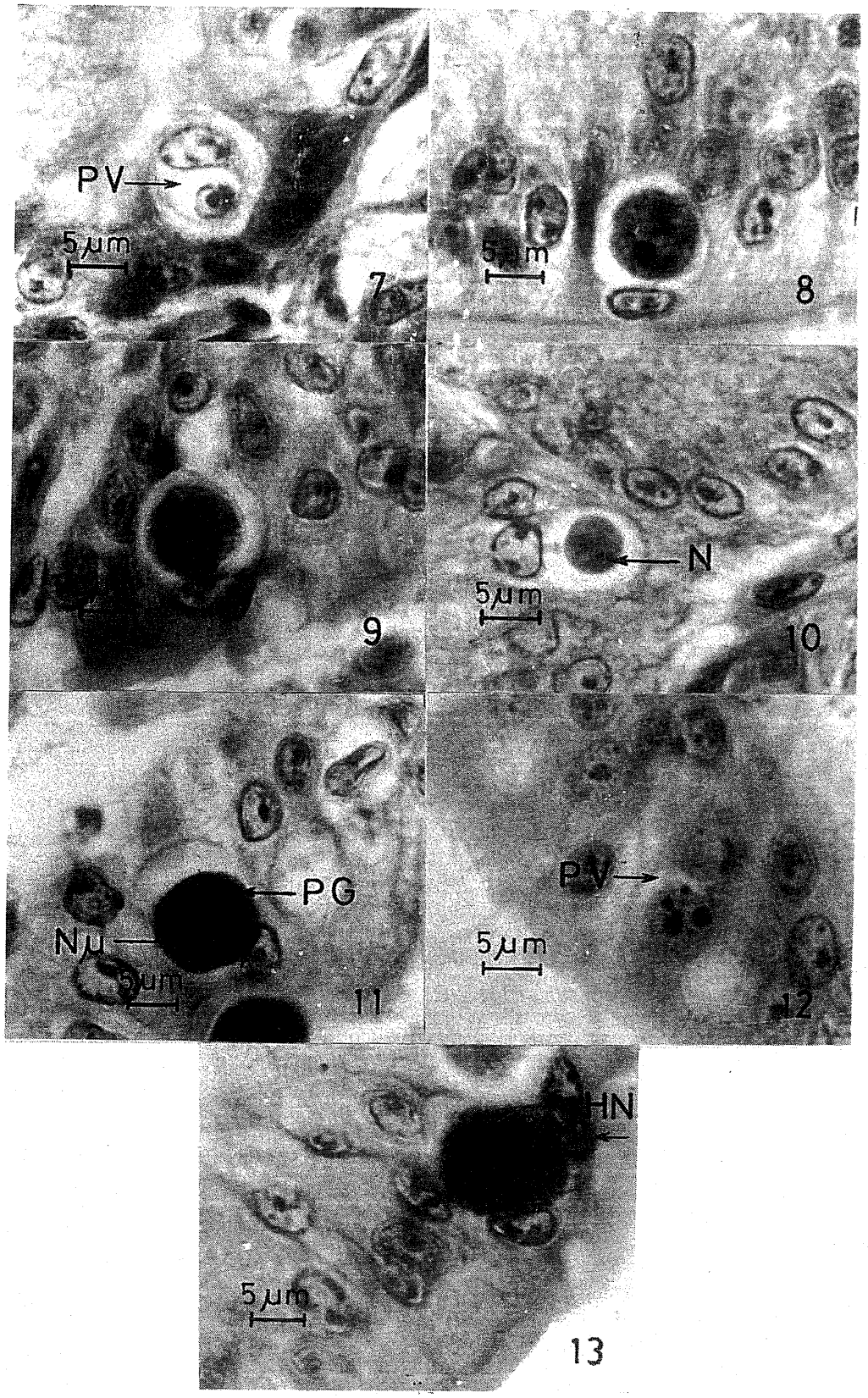
Fig. 2. Binucleate schizont with nuclei visible at margin; note the refractile globule (arrow).

Fig. 3. Immature 1st generation schizont with four peripheral nuclei around the refractile globule (arrow).

Fig. 4. First generation schizont with five mature merozoites, leaving a mass of residual cytoplasm (arrow).

Fig. 5. Second generation schizont with immature merozoites, budding from a central mass of residual body.

Fig. 6. Second generation schizont with four mature merozoites attached to a residual body (arrow).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refractile globules (Fig. 2) were first seen at 20 h. Immature 1st generation schizonts with refractile globules were also seen at 20 h (Fig. 3). Further nuclear division was accompanied by an increase in size.

First generation schizonts containing mature merozoites were first seen at 60 h after inoculation. Cytoplasmic division began on one side of the body separating the merozoites into bunches like bananas, leaving a mass of residual cytoplasm on one side (Fig. 4). Such schizonts were 12.5-14.5×7-9 μm (N=14). Mature merozoites were 7-9×1.9-2 μm. Because merozoites were usually arranged randomly in schizonts, their exact numbers could not be determined. However, 4 to 6 merozoites were thought to be present in schizont. Each merozoite had a central nucleus.

Second generation schizonts developed differently; the merozoites formed as buds from a central residual body. Schizonts containing immature merozoites were first seen 80 h after inoculation. They were 5.5×5.5 μm, (N=16) (Fig. 5). Schizonts containing mature merozoites were also first seen 80 h after inoculation. They were 12.5-14×7-8.5 μm (N=13) (Fig. 6). Mature merozoites were 6.5-7.5×2-2.5 μm (Fig. 6). There were 4 or 5 merozoites. They usually laid lengthwise within the schizonts. The nuclei of the merozoites, which were in one plane in stained sections of schizonts, were situated in the middle of the merozoites. Mature second generation schizonts had a residual cytoplasm which was much smaller than in the mature first generation schizonts (Figs. 4 and 6).

Gametogony

No sexual stages were seen in tissue sections until 80 h after inoculation when immature macrogamonts were observed and recognized by the relatively large nucleus and prominent nucleolus (Fig. 7). Macrogamonts with plastic granules developed were also seen (Figs. 8 and 9); they became easily recognizable as the plastic granules developed towards maturity (Fig. 11). Immature microgamonts were also discernible at the same time by their two deeply stained nuclei, and frequently by a slighly basophilic cytoplasm (Fig. 10). At 100 h after inoculation, mature macrogamonts and microgamonts were seen. Developing macrogametocytes were almost spherical (10.5-12.5×9-11 μm) (N=18) (Fig. 11). Mature macrogametocytes were characterized by a nucleus which contained a large nucleolus surrounded by a clear area. The strongly basophilic cytoplasm contained eosinophilic plastic granules at the periphery. Immature microgamonts were spherical with slightly enlarged nuclei that distinguished them from the small nuclei of the developing second generation schizonts and also by their pale basophilic cytoplasm (Fig. 12). The nuclei of microgametocytes (Fig. 13) became strongly basophilic as the micro-

Fig. 7. Immature macrogamont with large nucleus and nucleolus; note the parasitophorous vacuole (arrow).

Figs. 8 and 9. Macrogametocytes with developing plastic granules.

Fig. 10. Immature microgamont with two nuclei (arrow); note the lightly basophilic cytoplasm.

Fig. 11. Macrogametocyte with periphary plastic granules (arrow) stained lightly with eosin Y; note the large nuleosis (arrow) surrounded by a clear area.

Fig. 12. An early microgamont that appears partly to be free in the parasitophorous vacuole (arrow); note the deeply stained nuclei.

Fig. 13. Microgametocyte with strongly basophilic nuclei; nuclei; note the location and the host nucleus (arrow).

gamonts increased in size. Mature microgametocytes measured 12-14.5×10-11 μm (N=15). Microgametes in the tissues were difficult to find. Oocysts developed at 120 h after inoculation. They were unsporulated when passed.

The mean prepatent period was found to be 4.5 days and the patent period varied from 5 to 6 days in the five birds examined. The peak oocyst output in all 5 of the birds occurred on the 3rd day of the patent period (Fig. 14).

Periodicity of oocysts excretion-Oocyst output was examined daily from 3 to 6 h intervals. The shedding of oocysts happened between 15:00 and 21:00. Therefore, schizogony occurred during the night while gametogony occurred during daylight hours.

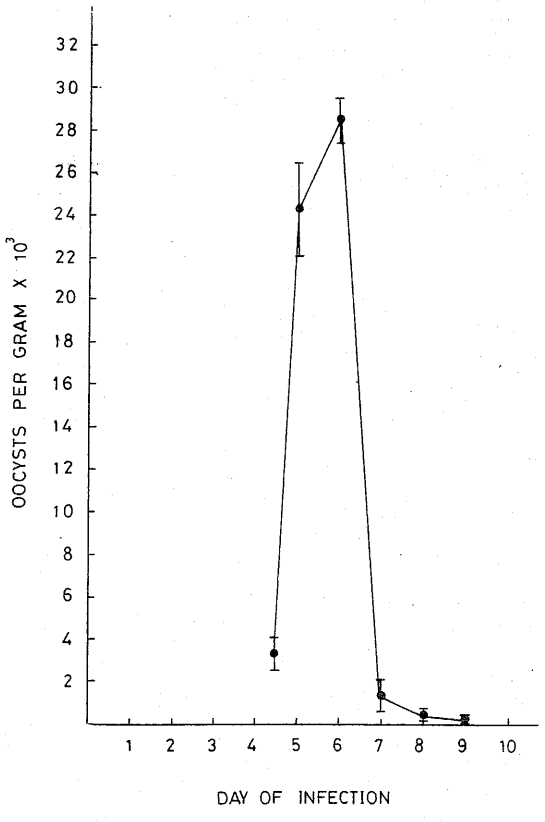

Fig. 14. Mean daily oocysts output of *Isospora elmahalensis* per gm feces during patent period. Each point is the mean±SEM of five replicants.

DISCUSSION

The endogenous stages of only one other species of *Isospora* from bulbuls is known and these are those of *I. pycnonotus* described by Mandal and Chakravarty (1964). Levine (1982) stated that "a coccidian species may be transmissible from one species to another in the same genus, but not from one genus to another in the same family unless otherwise demonstrated". According to Mandal and Chakravarty (1964) the endogenous stages of *I. pycnonotus* were represented by spherical schizonts which measured about 30×24 μm, and fully developed schizonts had 8 to 12 merozoites. In the present study, it was found that the developed schizonts of *I. elmahalensis* had only 4 to 6 merozoites. The microgametocytes of *I. pycnonotus* were slightly smaller than those of *I. elmahalensis* but the macrogametocytes were considerably larger with a more distinct membrane than *I. elmahalensis*. Because of the brevity of their description; the life cycles of the two species cannot be compared and it seems that only one generation of schizonts occurs in *I. pycnonotus*. The larger merozoites (8 and 7 μm in first and second generation schizonts, respectively) of *I. elmahalensis* are also distinctive from the short merozoites of *I. pycnonotus* (3.3 μm).

The prepatent period of *I. elmahalensis* was found to be 4.5 days and the patent period in acute infections varied from 5 to 6 days in the five birds examined. In contrast, Anwar (1966) stated that the prepatent period of *I. lacazei* was 4 days and the patent period in chronic infections was 30 days. He did not give figures on *I. chloridis*. In a separate study, Box (1977) found that the prepatent period of *I. serini* was 9-10 days and the patent period was 95 days. She attributed this to its asexual generations which took place in both extraintestinal tissues

(mononuclear phagocytes, MP) and the intestinal tissues, enabling this parasite to survive and produce infective forms for several months. The short patent period of *I. elmahalensis* is presumably linked with the potentially pathogenic nature of this parasite; however, it was necessary to conclude that several species examined in similar circumstances did not have an extened patent period and this was found to be so for *I. canaria* (Box, 1977) and *I. suis* (Biester and Murray, 1934; Vetterling, 1965).

The diurnal periodicity of oocyst discharge has been reported from a number of passerine birds (Boughton, 1933; Pellerdy, 1965; Kheysin, 1972; Stabler and Kitzmiller, 1972 and Box, 1977). In the case of *I. elmahalensis*, however, oocyst excretion increased in the afternoon of the 5th day after inoculation, very few oocysts were detected in the feces during the day from 10 AM but the fecal sample revealed that the oocyst output was primarily between 3 and 9 PM in the afternoon, similar to that described for *I. petrochelidon* (Stabler and Kitzmiller, 1972).

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球形孢子蟲 (*Isospora elmahalensis*) 寄生於白頰鴨 (*Pycnonotus leucogenys*) 之生活史

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本文描述球形孢子蟲 (*Isospora elmahalensis*) 寄生於白頰鴨 (*Pycnonotus leucogenys*) 的生活史。球形孢子蟲有兩個無性世代。成熟的第一世代裂殖體發現於感染後 60 小時，成熟的第二世代則發現於感染後 80 小時。有性世代的成熟大配子體細胞內具有顆粒發現於感染後 100 小時，且成熟的小配子體也同時出現。平均開放前期爲 4.5 日而開放期則爲 5～6 日。於開放期的第 3 天爲卵子出現最多的時候。五隻白頰鴨以卵孢母細胞餵食感染，發現卵孢子於第 5 日的下午 15:00～21:00 破裂釋出。