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Full Length Research Paper

Endogenous stages of *Isospora acanthodactyli* from sandy fringed-toed lizard (*Acanthodactylus schmidti)* in Al-Qassim, Saudi Arabia

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Endogenous stages of *Isospora acanthodactyli* from sandy fringed-toed lizard (*Acanthodactylus schmidti*) were described for the first time, in Al-Qassim, Saudi Arabia. Merogony and gamogony took place inside the host cell's cytoplasm, in the distal part of the small intestine. Multinucleated meronts, microgamonts and macrogametes were described and measured.

Key words: *Isospora acanthodactyli,* sandy fringed-toed lizard, Saudi Arabia, endogenous stages, Apicomplexa.

INTRODUCTION

Members of the genus *Isospora* have a single host. Merogony and gametogony take place within the host cells, and sporogony occurs outside the body. The oocysts contain two sporocysts, each containing four sporozoites (Mehlhorn, 1988). Many species of *Isospora* were described from lacertilian hosts (Finkelman and Paperna, 2002; Daszak et al., 2009). Regarding genus *Acanthodactylus*, *Isospora acanthodactyli* and *Isospora abdullahi* were described from *A. boskianus* (Sakran et al., 1994; Modry et al., 1998). In 1997, Al-Yousif and Al-Shawa described a new *Isospora* species infecting the sandy fringed-toed lizard (*A. schmidti*) in Saudi Arabia and was named *I. acanthodactyli*. Their study was only restricted to the exogenous stages (unsporulated and sporulated oocysts). To date nothing was published about the site of infection and endogenous development of the parasite, which is of paramount importance in order to place the coccidian parasite in its correct genus and species (Mehlhorn, 1988; Lainson and Paperna, 1999; Lainson et al., 2008). Therefore, it is necessary to determine the site of infection and describe the mode of endogenous development of the parasite, which we tried to achieve in this present work.

Abbreviations: SP, Sporocyst; **OL,** outer layer; **IL,** inner layer; **St,** stieda body; **RB,** refractile body; **ME,** multinucleated meront; **MC,** macrogamete; **NU,** nucleus; **MIC,** microgamont; **PV,** parasitophorous vacuole; **OC,** oocyst.

MATERIALS AND METHODS

A total number of 41 sandy fringed-toed lizards (*A. schmidti*) were captured from Al-Qassim area, Saudi Arabia, during 2008, and transferred to the laboratories of Faculty of Sciences and Arts, in Al-Rass governorate. Lizards were kept individually in plastic cages. Each one was dissected and faeces from the rectum, macerated in water, were examined for coccidian oocysts. Oocysts were collected by floatation technique method as described by Long et al. (1976). They were preserved in aqueous potassium dichromate to sporulate and the coccidium present was identified.

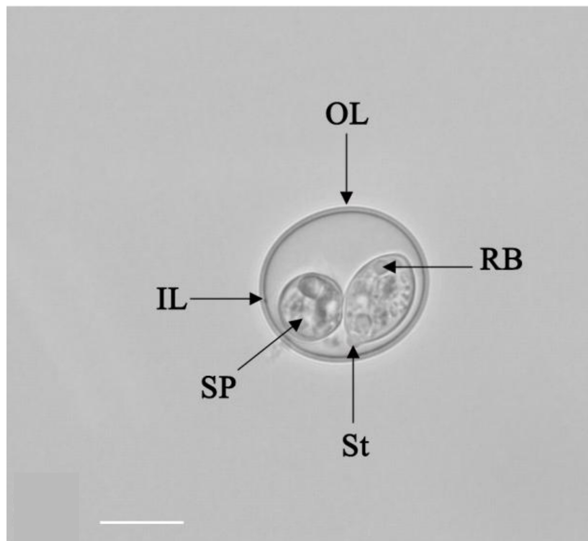

Figure 1. Subsperical, sporulated oocyst of *Isospora schmidti,* with two sporocysts (SP) and double-layered oocyst wall, outer layer (OL) and inner layer (IL). Sporocysts appeared with Steida bodies (St), Sporozoites with refractile body (RB). (Scale line = 10 µm).

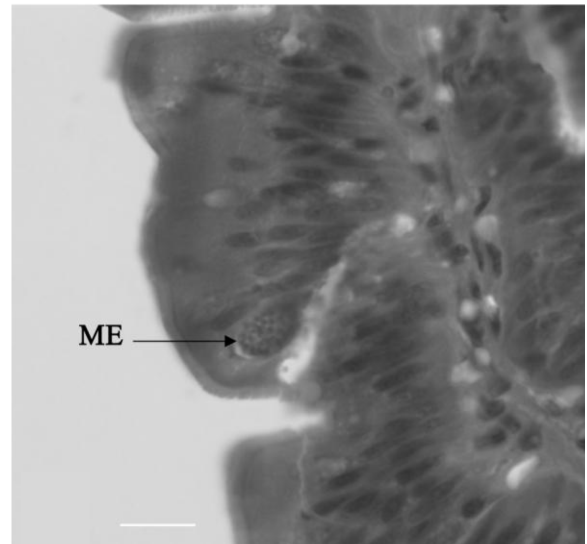

Figure 3. Endogenous development of *Isospora acanthodactyli* from *Acanthodactylus schmidti*. (Scale line = 10 µm). Multinucleate meront (ME), with many nuclei distributed in its cytoplasm.

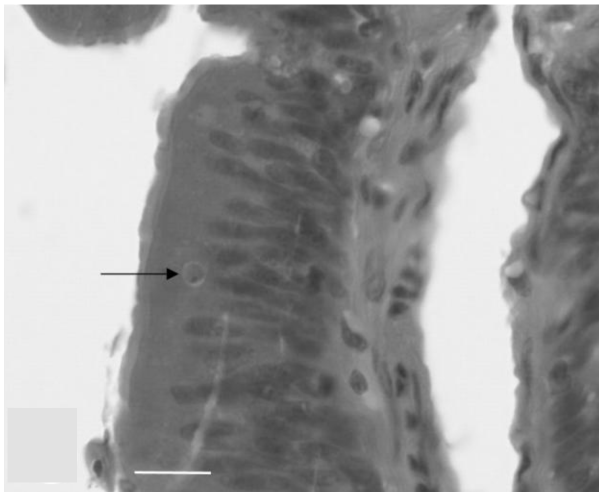

Figure 2. Endogenous development of *Isospora acanthodactyli* from *Acanthodactylus schmidti*. (Scale line = 10 µm). A small stage of parasite, presumably, uninucleate meront surrounded by parasitophorous vacuole (arrow).

To determine the site of infection, infected alimentary canals were removed, cut into pieces and the mucosa of each piece was scratched and examined. Parts of the liver and gall-bladder were examined as well. The infected part was processed by routine technique. Sections stained with haematoxylin and eosin were examined and photographed with a photomicroscope (Olympus BX53, Japan) (Al-Nasr, 2003).

RESULTS

Prevalence

It was found that the percentage of infection was 10% (4/41).

Morphology

Oocysts (OC) were sub-spherical, measured 28 × 25.5 µm. The L/W ratio was 1.1 (1 to 1.2). They were surrounded by smooth double-layered wall, lack micropyle, oocyst residuum and polar granules. Sporocysts (SP) were ovoid measured 14.1 × 10 (13.5 to 15 × 9 to 10.5) µm. Steida (St) and substeida bodies were present. Dispersed, numerous small, rounded granules which represent sporocyst residuum were also present. Each sporocyst was found to contain four sporozoites (Figure 1). Multinucleated meronts (ME) were elongated, large in size and contained slightly large nuclei (NU) which were scattered in the cytoplasm. They measured 10 × 7 (9 to 11 × 6 to 8.5) µm (Figures 2 and 3). Microgamonts (MIC) were recognized by a large number of small nuclei, arranged at the periphery of their cytoplasm, and measured 11 × 9 (10.3 to 11 × 8.1 to 9.4) µm (Figure 5). Macrogametes (MC) were large in size and they were sub-spherical and contained two types of wall-forming bodies, arranged on the periphery of cytoplasm. Their nuclei were centrally located. They measured 10.5 × 9 (9.7 to 11 × 8.8 to 9.4) µm (Figure 4). Mature zygotes, filled with amylopectin were observed. They measured 16 × 11 (15.5 to 16.2 × 10.5 to 11.7) µm (Figure 6).

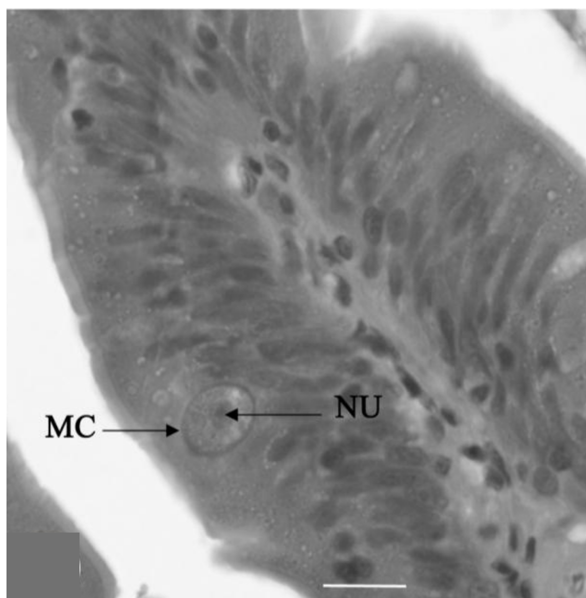

Figure 4. Endogenous development of *Isospora acanthodactyli* from *Acanthodactylus schmidti*. (Scale line = 10 µm). Macrogametes (MC) with a central nucleus (NU) and wall-forming bodies at the periphery of its cytoplasm.

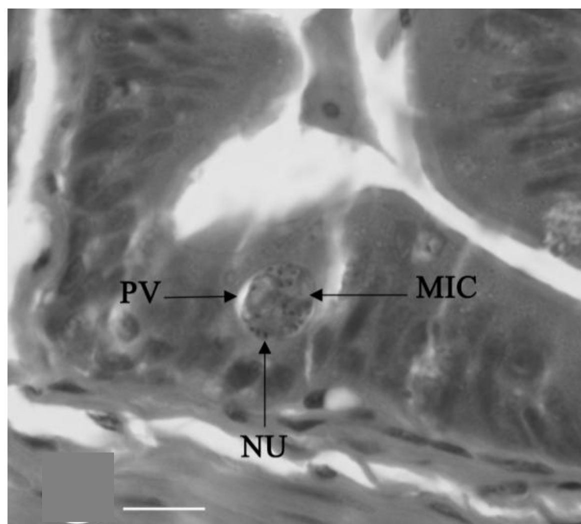

Figure 5. Endogenous development of *Isospora acanthodactyli* from *Acanthodactylus schmidti*. (Scale line = 10 µm). Microgamonts (MIC) with many fine nuclei (NU) surrounded by parasitophorous vacuole (PV).

Site of infection

Merogony and gamogony occurred in the cytoplasm of the host epithelial cells of the small intestine.

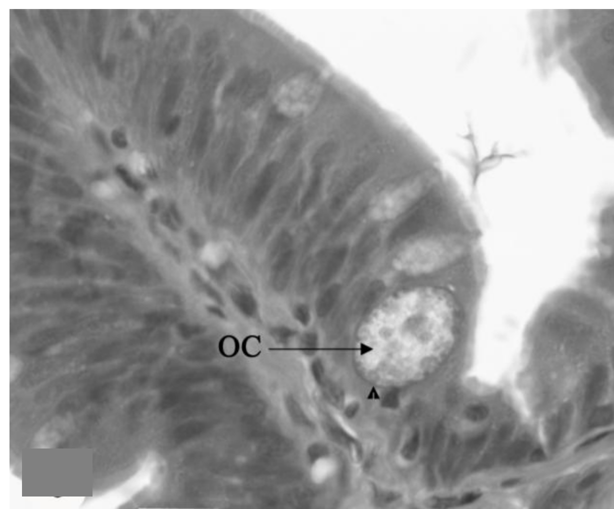

Figure 6. Endogenous development of *Isospora acanthodactyli* from *Acanthodactylus schmidti*. (Scale line = 10 µm). Oocyst (OC) filled with amylopectin, arrowhead showed newly-formed oocyst wall.

DISCUSSION

In the present work, the same description and measurements of oocysts, presence of oocyst residuum, steida and substeida bodies and sporocyst residuum were reported by Al-Yousif and Al-Shawa (1997). These descriptions similarities indicated that the parasite is *I. acanthodactyli.* Identification of a large number of recorded *Isospora* species have been based only on the morphological features of their oocysts, sporocysts and sporozoites, however further investigations concentrated on mode of development inside the hosts revealed that the parasites of reptilian hosts with oocysts of isosporan morphology are divisible into two distinct groups: those whose endogenous stages are intranuclear and those whose endogenous stages are in the host-cells cytoplasm.

Peculiarities of the endogenous development of reptilian coccidians previously included in the genus *Eimeria*, have led to their allocation into the genera *Choleoeimeria* and *Acroeimeria* (Paperna and Landesberg, 1989). Similarly, separation of isosporean species into two groups might occur according to development within their host cells (intranuclear or intracytoplasmic).

In the present paper, merogony and gamogony occurred within the host-cell cytoplasm at the distal end of the epithelial cells of the small intestine, this agreed with the *Isospora* species which develop in their host-cell cytoplasm, for example *I. ameivae* and *I. chalchidis*, but disagreed with the intranuclear species, *I. hemidactyli*, *I. stenodactyli* and other isosporeans developing within the

nuclei of their host cells (El- Toukhy et al., 1994, 1996; Lainson and Paperna, 1999; Paperna and Lainson, 2000). Multi nucleate meronts (premature meronts) contained up to 15 nuclei, in contrast to six nuclei seen in cross sections of *I. hemidactyli* infecting the gecko *Hemidactylus mabouia* in north Brazil, and *Isospora carliae* infecting skink *Carlia rhomboidalis* in Australia (Paperna, 2006).

Young microgamonts of the present study had small, compact and densely stained nuclei arranged on the surface of the parasite cytoplasm which agreed with the descriptions of the same stages of *I. ameivae* (Lainson and Paperna, 1999). Macrogametes had the normal shape and structure of most other intracytoplasmic described *Isospora* species. Generally, the description of the developing stages of *I. acantodactyli* in the cytoplasm of the gut-epithelial cells of *Acanthodactylus shmidti* conforms with the same stages observed in other intracytoplasmic species of *Isospora* described from other saurian hosts. The importance of this work is to complete the biological information of the parasite. This will facilitate to put the present *Isospora* species in its exact situation when the *Isospora* species are separated, in the future, into intracytoplasmic and intranuclear species.

Conclusion

I. acanthodactyli infections are present in sandy fringed-toed lizards in this region, as determined by examination of their faeces. Endogenous stages of this parasite occurred in the cytoplasm of the host epithelial cells of the small intestine. This parasite needs to be further studied to determine their life cycle, by using experimental infection.

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