



***ISOSPORA TAIZII* (APICOMPLEXA: EIMERIIDAE), A NEW
COCCIDIAN PARASITE FROM THE YEMEN CHAMELEON
(*CHAMELEON CALYPTRATUS*) (SAURIA: CHAMAELEONIDAE)
IN TAIZ CITY, YEMEN REPUBLIC**

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ABSTRACT :

Isospora taizii n. sp. was isolated from the faeces of the Yemen chameleons (*Chameleon calyptratus*). Sporulated oocysts of *Isospora taizii* n. sp. were subspherical to ovoid 28 (25-32) × 22 (18-26) μm. The oocyst wall is bilayered, smooth and colorless approximately 1.2 μm thick. The outer layer is significantly thicker than the inner one. Micropyle and oocyst residuum are absent. Sporocysts are ellipsoidal, 12.8 (14-15) × 9.2 (10-12) μm. the sporocyst wall is single-layered, thin, smooth and colourless, with a small stieda body at the pointed end of the sporocyst. Sporozoites are elongate, lying length-wise in the long axis of the sporocyst. Fully sporulated oocysts are observed within 48 hours at 26°C. When compared this parasite with other types of *Isospora* sp infecting Chameleon were found a new species and named *Isospora taizii* according to the name of locality, Taiz Governorate,

INTRODUCTION:

Coccidial parasites of the genus *Isospora* (Apicomplexa: Eimeriidae) are the cause of intestinal disease, widely distributed among reptiles (Amoudi, 1993; Modry *et al.*, 1998; Paperna & Lainson, 2000 and McQuisston *et al.*, 2001), Family: Chamaeleonidae (Modry & Koudela, 1995; Modry *et al.*, 1997 & 1999 & 2000 and 2001), birds (Ball & Daszak, 1997 and Upton *et al.*, 2001), mammalian host species (Sayd & Kawazoe, 1998 and Mundt *et al.*, 2003) and on humans which were given recently by (Frenkel *et al.*, 2003 and Resiere *et al.*, 2003).

Coccidia infection causes a watery diarrhea, which is sometimes bloody. These protozoan

parasites have asexual and sexual stages within the intestinal cells of their hosts and produce an environmentally resistant cyst stage, the oocyst. Infections are acquired by the ingestion of infective stage (sporulated oocysts) in contaminated food or water. The sporulated oocyst breaks open and release eight sporozoites. This sporozoite each finds an intestinal cell and begins to reproduce inside it. Ultimately, the cell is so full of what are called "merozoites" that it bursts releasing the merozoites, which seek out their own intestinal cells, and the process begins again. It is important to note how thousands of intestinal cells can become infected and destroyed as a result of accidentally swallowing a single oocyst.

Only one species of *Isospora* was described from saurian hosts in Yemen chameleon by Modry & Koudela (1995). This paper describes the oocysts of *Isospora taizii* n. sp. from the faeces of the Yemen chameleon *Chameleon calypttratus* (Sauria: Chamaeleonidae), and compared their structure to those reported from other members of this family.

MATERIALS AND METHODS:

The Yemen chameleons *Chameleon calypttratus* were captured alive. This animal is a large, aggressive species, the veiled chameleon occupies the wadis and agricultural lands of this otherwise arid region. The nominate form, *Chameleon calypttratus* is found in the more southern reaches of the distribution in Yemen and southwestern Saudi Arabia (Le Berre, 1994 and Barnett *et al.*, 1999).

The alimentary canal of each animal was opened. Smears from the intestinal contents were prepared from successive parts and examined microscopically. For determination of the sporulation time, oocysts were collected from faeces and strained through sieve. After staining, the suspension including the water used in washing the sieve, was allowed to undergo sedimentation after which the water was siphoned off. The sediment was then suspended in 2.5% Potassium dichromate solution ($K_2Cr_2O_7$) to sporulate. The suspension was then spread in a thin layer in petri dishes and kept at room temperature 25-28°C for sporulation. Sporulated oocysts were examined with a Zeiss universal photomicroscope with 100X Planapochromatic oil immersion objective lenses. The oocysts were measured with ocular micrometer. All measurements are in μm and given as means, followed by the range in parentheses and the shape-index (ratio of length /width).

RESULTS:

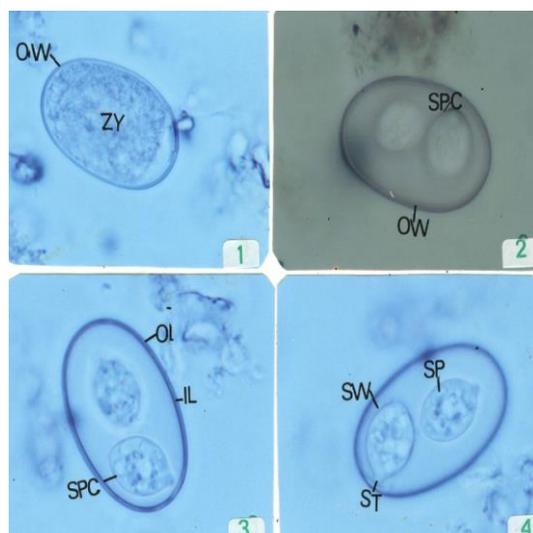
During a limited survey for coccidian infections in some of the reptiles in Taiz region between March and July 2003, faeces samples of 28 Yemen chameleons were examined by cover slip floatation with Sheather's solution (Levine, 1973). From the 28 Yemen chameleon, 18 animals (64.3%) were infected with *Isospora taizii* n. sp. detailed examination of sporulated oocysts revealed apparently new species of *Isospora*, which does not resemble either any other species described from chameleons or published descriptions of any species of *Isospora* found in other chameleon.

Examination of freshly shedded, non-sporulated oocysts in the faeces collected from several individuals show the cytoplasm of the zygote was containing large granules mostly at the periphery and smaller ones in the center. No nucleus could be seen in freshly passed oocysts (Fig. 1). These oocysts were subspherical to ovoid in shape, measured 28 (25-32) μm in length and 22 (18-26) μm in width. The oocyst wall is bilayered, smooth and colourless approximately 1.2 μm thick. The outer layer was light and thin, while the inner layer was dark and thick.

No micropyle or polar granules were observed. At the beginning of the sporulation process, the cytoplasm condensed (Fig. 2), divided, and two sporoblasts were formed without leaving an oocyst residuum. Sporoblasts developed forming sporocysts. As sporulation proceeded, four sporozites developed within each sporocyst (Figs. 3&4). Later on, the sporoblasts begin to elongate with the formation of sporocysts. These sporocysts were ellipsoidal in shape. Then, differentiation of sporozoites took place (Fig. 4). The sporocysts measured 14-15 μm in length and 10-12 μm in width, with a mean length \times width 12.8 \times 9.2 μm . The sporocyst wall is single layered, thin, smooth and

colourless. A sporocyst residual body and a steida and substeida bodies were present. Each sporozoite contains spheroid anterior refractiel body and spheroid or ellipsoid posterior

refractiel body, nucleus located between the refractile bodies. Fully sporulated oocysts are observed within 48 hours at 26°C.



Explanation of Figures

Figs. (1-4): Photomicrograph for events of sporulation process for the subspherical to ovoid oocysts *Isospora taizii* n. sp. All photos. × 1600.

Fig. (1): Fresh non-sporulated oocyst with zygote (ZY) nearly filling the space of oocyst. The oocyst is enclosed with bilayered wall, outer layer (OL) and the inner layer (IL) the oocyst wall (OW).

Fig. (2): Two sporoblasts (SB) are found each with four sporozoites.

Figs. (3,4): Full sporulated oocysts of *Isospora* with two sporocysts (SPC) each with four sporozoites (SP). Notice: residual body (RB) in sporocysts, Steida body (ST) and substeida body in the sporocyst. Residuum bodies absent in the oocysts but present in the sporocysts.

Taxonomic summary of *Isospora taizii* n. sp. :

Host type: Yemen chameleon (*Chameleon calyptratus*) Sauria: Chamaeleonidae.

Location type: Taiz Governorate, Yemen Republic.

Prevalence: found in 18 of 28 (64.3%).

Sporulation time: 48 hours at 26°C.

Site of infection: small and large intestine.

Sporulation: Exogenous oocysts were passed unsporulated and fully sporulated oocysts were observed within 48 hours at 26°C in 2.5% K₂Cr₂O₇.

Etymology: The specific name is derived from the collection locality, Taiz Governorate.

DISCUSSION:

About 65 named *Isospora* species have been described to date from saurians. Only 6 *Isospora* spp. were described from the Family: Chamaeleonidae (Chameleons) (Duszynski and Couch, 2003). *Isospora mesnili* from *Chamaeleon chamaeleon* (Sergent, 1902); *Isospora jaracimrmani* from *Chamaeleon calyptratus* (Modry and Koudela, 1995), *Isospora tigris* from *Calumma tigris* (Tiger

chameleon) (Modry *et al.*, 1997); *Isospora wildi* from *Chamaeleon dilepis* (Flap-necked chameleon) (Modry *et al.*, 2000); *Isospora munriyu* from *Chamaeleon jacksonii* (Jackson's chameleon) (Modry *et al.*, 2000) and *Isospora necasi* from *Chamaeleon melleri* (Meller's chameleon) (Modry *et al.*, 2000). However, Modry *et al.*, (1999) described one, *Isospora chamaeleolidis n. sp.*, from *Chamaeleolis barbatus* also, Modry *et al.*, (2001) described one *Isospora brygooi n. sp.*, from five new coccidian species infecting Madagascar chameleons of the genera *Furcifer* and *Brookesia*.

Although, the oocyst shape of *Isospora taizii n. sp.* is subspherical to ovoid only similar with *Isospora wildi* and *Isospora necasi* (Modry *et al.*, 2000). The absence of oocyst residuum body and the ellipsoidal shape of the sporocyst in the present study was similar to *Isospora wildi*; *Isospora munriyu* and *Isospora necasi* (Modry *et al.*, 2000) and *Isospora brygooi* (Modry *et al.*, 2001). In addition, the presence of sporocyst residuum, Stieda and substieda bodies was similar to four *Isospora* species from the chameleon (*Isospora wildi*; *Isospora muriyu* and

Isospora necasi (Modry *et al.*, 2000) and *Isospora brygooi* (Modry *et al.*, 2001). However, the primary differences center on the size of the oocyst, *Isospora taizii* 28 (25-32)× 22 (18-26) µm, but in *Isospora Isospora chamaeleolidis* 16.1(13-21)×15.6 (13-19) µm (Modry *et al.*, 1999) ; *Isospora wildi* 25 (22-28)× 21.4 (18-24) µm. ; *Isospora necasi*. 26.6 (21-30)×24 (20-27) µm. and *Isospora muriyu* 23 (21-25)× 21.9 (21-23) µm (Modry *et al.*, 2000), and *Isospora brygooi* 20.7 (17-24.5)× 19.3 (16-23) µm (Modry *et al.*, 2001). Although, the surface of oocyst wall of the *Isospora taizii* is smooth double layer 1.2 µm thick; that of the oocyst of *Isospora chamaeleolidis* bilayered 1.0-1.5 µm thick; *Isospora necasi* is a velvetlike wall 2 µm; *Isospora munriyu* is a finely granulated wall 1.5 µm thick; *Isospora brygooi* is a slightly pitted wall, and *Isospora wildi* is a smooth wall 1 µm thick. So, it is obvious that the *Isospora* species found in the present host genus *Chamaeleon calyptratus* belong to a new species and it is suggested to be named *Isospora taizii* according to the locality.

Comparative data of *Isospora sp.* from Family: Chamaeleonidae (Chameleons)

<i>Isospora</i> Species	<i>Isospora taizii</i>	<i>Isospora chamaeleolidis</i>	<i>Isospora wildi</i>	<i>Isospora munriyu</i>	<i>Isospora Necasi</i>	<i>Isospora brygooi</i>
Type host	<i>Chamaeleon calyptratus</i>	<i>Chamaeleolis barbatus</i>	<i>Chamaeleo dilepis</i>	<i>Chamaeleon Jacksonii</i>	<i>Chamaeleon melleri</i>	<i>Furcifer pardalis</i>
Oocyst Shape	Subspherical to ovoid	Spherical to subspherical	Subspherical to ovoid	Spherical to subspherical	Subspherical to ovoid	Spherical to subspherical
Oocyst length/width	28 (25-32) x22 (18-26) µm	16.1(13-21) x 15.6 (13-19) µm	25 (22-28) x 21.4 (18-24) µm	23.6 (21.5-25) x 21.9 (21-23) µm	26.6 (21-30) x 24(20-27) µm	20.7(17-24.5) x 19.3 (16-23) µm
Oocyst wall	Smooth double layer 1.2 µm	Bilayered 1.0-1.5µm	Smooth 1µm	Granulated 1.5µm	Velvet like 2 µm	Slightly pitted wall
Sporocyst shape	Ellipsoidal	Ellipsoidal	Oval to ellipsoidal	Broadly ellipsoidal	Ellipsoidal	Ellipsoidal
Sporocyst Length /width	12.8 (14-15) X 9.2 (10-12) µm	10.8 (10-13) X 7.8 (7-9) µm	12.3 (12-13) X 9.7 (9-10) µm	12.4 (12-13) X 8.7 (8-10) µm	12.8 (12-14) X 9.8 (9-10) µm	12.2 (11.5-13) X 8.1 (8-8.5) µm
Locality	Taiz, Yemen	Cuba	East African	East African	East African	Madagascar
Reference	Abdel-Wasae present report	Modry <i>et al.</i> , (1999)	Modry <i>et al.</i> , (2000)	Modry <i>et al.</i> , (2000)	Modry <i>et al.</i> , (2000)	Modry <i>et al.</i> , (2001)

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وصف نوع جديد من الطفيليات الأولية (ايزوسبورا تعيزي) التابع لعائلة إيميريا المتطفل على الحرباء اليمنية كاملون كالبيتراتس التابع لعائلة العظائات في محافظة تعز- الجمهورية اليمنية

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بفحص ٢٨ من الحرباء اليمنية جنس كاملون كالبيتراتس من محافظة تعز وجد إنها مصابة بنوع جديد من طفيليات الكوكسيديا التابع لجنس الايزوسبورا. وقد جمعت بويضات هذا الطفيل من الفضلات في الأمعاء الدقيقة والغليظة. وقد دل الوصف الدقيق إن الكيس الجرثومي لهذا الطفيل شبة مستدير إلى بيضاوي الشكل، ويتراوح قطره من ٢٢ (١٨-٢٦) - ٢٨ (٢٥-٣٢) ميكرون، كما أن له جدارين سمكهما ١.٢ ميكرون ولا يحتوي الكيس الجرثومي لهذا الطفيل على نقيير ولا حشوة سيتوبلازمية ولا على أقطاب حبيبة، ويوجد بداخل الكيس الجرثومي كيتين بوغيين لهما شكل أهليجي، وتتراوح أطوال كل منهما بين ٩.٢-١٢.٨ ميكرون. وللكيس البوغي استديا واضحة في مقدمة الكيس البوغي عند الطرف المدبب. وتتخذ الأبواغ الجرثومية في كل في كل كيس بوغي وضع طولي متعاكس بحيث يكون راس أحد البوغين مع ذيل البوغ الآخر، وتحيط بهما حشوة سيتوبلازمية من حبيبات كبيرة، كما تمت مقارنة هذا الطفيل الحالي بالطفيليات السابق وصفها من نوع الايزوسبورا التي سبق أن وصفها العلماء الآخرين. مما دعانا إلى اعتبارها مختلفة تماما واعتبرناها نوع جديد من الايزوسبورا التي تصيب الحرباء اليمنية، أطلق عليه اسم ايزوسبورا تعيزي إشارة إلى اسم المحافظة التي عثر عليه بها.