

## New species of *Choleoeimeria* (Apicomplexa: Eimeriidae) from the veiled chameleon, *Chamaeleo calypttratus* (Sauria: Chamaeleonidae), with taxonomic revision of eimerian coccidia from chameleons

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**Abstract.** Coprological examination of 71 samples from a breeding colony of veiled chameleons, *Chamaeleo calypttratus* Duméril et Duméril, 1851, revealed a presence of two species of coccidia. In 100% of the samples examined, oocysts of *Isospora jaracimrmani* Modrý et Koudela, 1995 were detected. A new coccidian species, *Choleoeimeria hirbayah* sp. n., was discovered in 32.4% of samples from the colony. Its oocysts are tetrasporocystic, cylindrical, 28.3 (25–30) × 14.8 (13.5–17.5) µm, with smooth, bilayered, ~1 µm thick wall. Sporocysts are dizoic, ovoidal to ellipsoidal, 10.1 (9–11) × 6.9 (6–7.5) µm, sporocyst wall is composed of two plates joined by a meridional suture. Endogenous development is confined to the epithelium of the gall bladder, with infected cells being typically displaced from the epithelium layer towards lumen. A taxonomic revision of tetrasporocystic coccidia in the Chamaeleonidae is provided.

In reptilian hosts, monoxenous coccidia, namely *Eimeria* Schneider, 1875 sensu lato and *Isospora* Schneider, 1881 represent commonly diagnosed protozoans of remarkable diversity (Barnard and Upton 1994, Greiner 2003). In addition to seven species of *Isospora*, there are eight *Eimeria* s.l. species (see Table 1) described from the Chamaeleonidae to date (Sergent 1902, Modrý and Koudela 1995, Modrý et al. 1997, 2000, 2001a, b). Four eimerian species, namely *Eimeria bohemiae*, *Eimeria largeni*, *Eimeria tilburyi* and *Eimeria hajeki*, originate from East African chameleons. Further four species of *Eimeria* have been described from Madagascar: *Eimeria glawi*, *Eimeria vencesi*, *Eimeria worthi* and *Eimeria brookesiae* (Modrý et al. 2001b). For authorship of the above-mentioned *Eimeria* species and their type hosts see Table 1.

The family Chamaeleonidae represents an ancient group of saurian reptiles, supposed to have biogeographic patterns associated with the Gondwanan break-up of Madagascar and Africa. The origin of the group is estimated to be more than 60 million years ago (Klaver and Böhme 1986, Hofman et al. 1991). More than 130 species of the Chamaeleonidae have been described to date and monophyletic origin of the family is supported by several studies (Klaver 1981, Hillenius 1986, Frost and Etheridge 1989). *Chamaeleo calypttratus* Duméril et Duméril, 1851, the object of present study, occurs at the southern tip of Arabian Peninsula, in Yemen and Saudi Arabia. As it easily reproduces in captivity, this species became very popular among herpetoculturists in past two decades and tens of thousands

of individuals are kept in North America and Europe (Nečas 2004).

Until now, *Isospora jaracimrmani* Modrý et Koudela, 1995 is the only eimerian coccidium described from *C. calypttratus* reported to cause serious health problems in captive animals (Modrý and Koudela 1995, 1998). Herein we describe a new, highly pathogenic species of *Choleoeimeria* Paperna et Landsberg, 1989 from a captive group of *C. calypttratus*, and provide taxonomic revision of the coccidia from lizards of the family Chamaeleonidae.

### MATERIALS AND METHODS

**Coprological examination.** In 2003, faecal samples of a group of veiled chameleons were examined (parental generation was imported from Yemen in 2002, being originally collected in the wild). Animals were kept by a private breeder in 50-litre hygienically designed plastic cages (females up to three individuals per cage, males individually). Samples were collected from all cages (n = 71), placed into plastic vials with 2.5% potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) and transported to the laboratory. All samples were examined, measured and photographed after concentration by flotation with a modified Sheather's sugar solution (s.g. 1.30), using an Olympus AX 70 microscope equipped for Nomarski interference contrast (NIC). Measurements are reported in micrometres (µm) as the mean, followed by the range in parentheses.

**Post mortem examination.** Five animals from a breeding group were examined. Two living moribund specimens were euthanized, the remaining three were freshly dead. All were

kept in a refrigerator for several hours prior the dissection. Gall bladder contents were examined as wet mounts and pieces of gall bladder mucosa as squash preparations.

Samples of the gall bladder and small intestine for histological examination were obtained from the two euthanized moribund animals, preserved in 10% buffered formalin, and processed routinely for histology using haematoxylin-eosin staining. Gross changes were documented using a digital camera. Intestinal contents of all animals were examined using Sheather's sugar flotation method as described above.

**Experimental trials.** Experimental infection of six young *C. calyptratus* (1 month old) was performed in order to obtain missing data about endogenous development and to confirm pathogenicity. Oocysts for inoculation originated from the gall bladder content of a dissected animal. Oocysts were flushed by repeated centrifugation and counted. All experimental animals were examined repeatedly prior the experiment and then inoculated by oesophageal tube. One animal (No. 1) received 60,000 oocysts, while the remaining five (Nos. 2–6) received 25,000 oocysts each. Animals were kept separately, in glass terraria at 22–28°C. Faecal samples were examined weekly, using the method described above. Dead or euthanized chameleons were dissected and examined as described above.

## RESULTS

### Coprological examination

The presence of two species of coccidia was found in examined samples from the breeding colony of *Chamaeleo calyptratus*. *Isospora jaracimrmani*, identified based on the oocyst morphology, was found in 100% (n = 71) of the cages examined.

The second species was identified as a member of the genus *Choleoeimeria*. Oocysts of this coccidium were detected in 32.4% (n = 71) of the samples examined. Comparison with all other tetrasporocystic coccidia described from chameleons (see Table 1) revealed that it is a new species, which is described below.

### Post mortem examination

In four dissected animals from the colony, dilatation of the gall bladder was recorded, with a white, macroscopically visible precipitate in the gall bladder content (Fig. 1). Microscopic examination of the precipitate confirmed that it was formed by a mass of oocysts of *Choleoeimeria* sp. at various stages of sporulation (Fig. 2). One dissected animal showed no gross pathomorphological lesions. These oocysts were absent in the intestinal content of two of four dissected animals, even though they had heavily infected gall bladder mucosa and masses of oocysts in their gall bladder contents.

Examination of the intestinal content revealed the presence of *Isospora jaracimrmani* oocysts in all five animals. Endogenous stages (mostly matured macrogamonts) of this coccidium were found in enterocytes of the small intestine.

## *Choleoeimeria hirbayah* sp. n.

Figs. 2–9

### Exogenous stages

Oocysts tetrasporocystic, cylindrical, 28.3 (25–30) × 14.8 (13.5–17.5), shape index (length:width ratio, SI) 1.92 (1.63–2.19) (Figs. 3, 9). Micropyle and polar granule absent. Oocyst wall smooth, bilayered, ~1 thick. Irregularly curved projections of folded inner layer of oocyst wall visible inside oocyst. Sporocysts dizoic, ovoidal to ellipsoidal 10.1 (9–11) × 6.9 (6–7.5), slightly irregular in shape and size, even within a single oocyst, sporocyst SI 1.48 (1.38–1.69). Sporocysts without Stieda body; wall composed of two plates joined by meridional suture. Sporocyst residuum present, composed either of 3–6 large, spherical globules 1.5–2 in diameter or present as cluster of fine granules. Sporozoites elongated, relatively robust, heavily granulated without visible striation, arranged head to tail within sporocyst, refractile bodies and nuclei not discernible (Fig. 9).

### Endogenous stages

In histological sections, endogenous stages (trophozoites, meronts, macrogamonts and microgamonts) were observed in the gall bladder epithelium of all four animals with gall bladder dilatation (Fig. 4). Infected epithelial cells were displaced from the mucosa towards the lumen, usually communicating with the basal membrane only by a thin pedicle (Fig. 4).

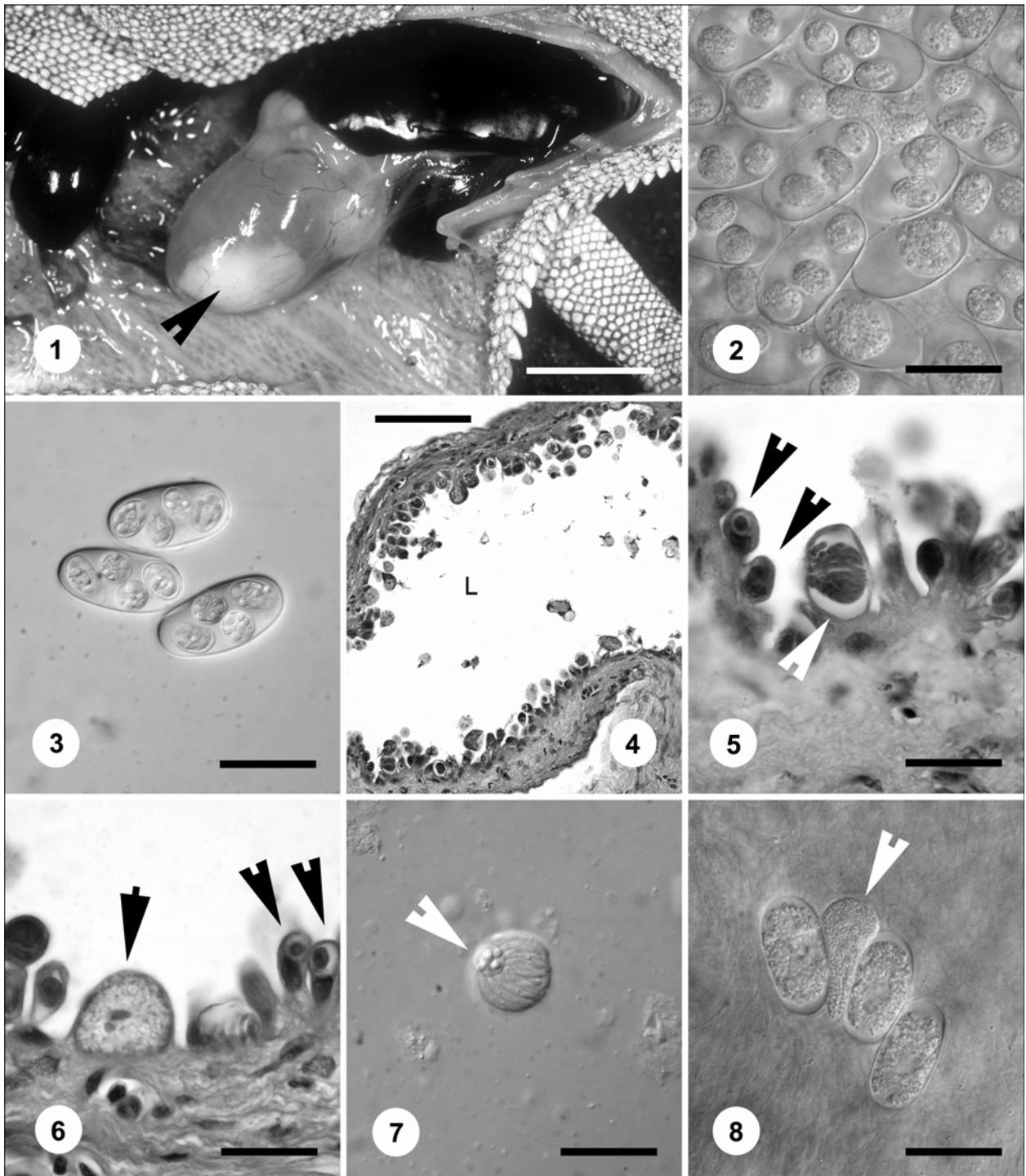
Meronts rounded to oval, 17.4 (15–22) × 14 (11–18), containing up to ~100 banana-shaped merozoites 7 × 1.5 (Figs. 5, 7). Macrogamonts oval, 15.1 (11–20) × 14.1 (10–16), with centrally located nucleus; nucleolus usually present (Figs. 6, 8). Wall-forming bodies present on the periphery of macrogamont cytoplasm. Microgamonts oval, 20 (17–23) × 17.6 (14–22) containing high number of elongated microgametes. Part of oocysts in the gall bladder of dissected animals was unsporulated, either containing spherical sporont filling most of the oocyst or being at various stage of sporulation.

### Experimental trials

Results of experimental infections with *Choleoeimeria hirbayah* oocysts confirmed localisation of the endogenous development in the gall bladder epithelium cells, histological findings in positive dissected animals being identical to those described above. Oocysts present in faeces in initial stages of shedding were unsporulated, with large sporont; the percentage of sporulated oocysts was higher as the infection became chronic and by ~4 months after inoculation, more than 90% of oocysts were fully sporulated.

### Taxonomic summary

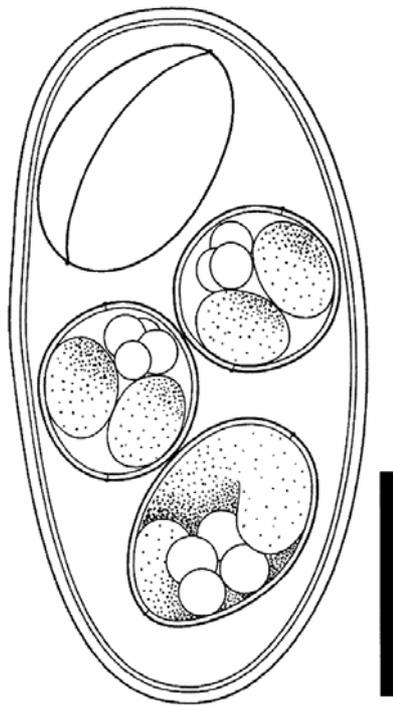
Type host: Veiled chameleon, *Chamaeleo calyptratus* Duméril et Duméril, 1851 (Sauria: Chamaeleonidae).



**Figs. 1–8.** Endogenous and exogenous stages of *Choleoeimeria hirbayah* sp. n. in infected *Chamaeleo calytratus*. **Fig. 1.** Dilatation of the gall bladder in dissected chameleon. Note distinct white coagulum formed by oocysts (arrowhead). **Fig. 2.** Oocysts at various stage of sporulation from the gall bladder content (NIC). **Fig. 3.** Sporulated oocysts from faeces (NIC). **Fig. 4.** Gall bladder mucosa with infected cells displaced from the epithelium towards the lumen (L); histological section, H&E staining. **Fig. 5.** Meront composed of numerous banana-shaped merozoites (white arrowhead) and undifferentiated trophozoites (black arrowheads); histological section, H&E staining. **Fig. 6.** Macrogamont (arrow) and trophozoites (arrowheads); histological section, H&E staining. **Fig. 7.** Rounded meront with merozoites and distinct residual body (arrowhead); squash preparation (NIC). **Fig. 8.** Group of three oocysts and single macrogamont (arrowhead); squash preparation (NIC). Scale bars: Fig. 1 = 10 mm; Figs. 2, 3 = 15  $\mu$ m; Fig. 4 = 100  $\mu$ m; Figs. 5–8 = 20  $\mu$ m.

**Table 1.** Comparative data on tetrasporocystic coccidia described from the Chamaeleonidae.

Species	Type host	Oocyst size (range) SI	Oocyst shape	Polar granules Oocyst residuum	Sporocyst size (range) SI	Localisation of endogenous development	Locality	Reference
<i>Choleoimeria bohemiae</i> comb. n.	<i>Chamaeleo melleri</i>	25 × 14 (24–26 × 13–15) SI = 1.79 (1.67–1.92)	cylindrical	absent absent	9.4 × 6.5 (9–10 × 6–7) SI = 1.46 (1.29–1.67)	unknown, oocysts in faeces	Tanzania	Modrý et al. 2000
<i>Choleoimeria largeni</i> comb. n.	<i>Chamaeleo gracilis</i>	31.2 × 19.3 (29.5–34 × 18.5–20) SI = 1.62 (1.48–1.79)	cylindrical	present (1–3) absent	10.2 × 7.6 (10–11 × 7–8.5) SI = 1.36 (1.18–1.5)	unknown, oocysts in faeces	Kenya	Modrý et al. 2000
<i>Choleoimeria tilburyi</i> comb. n.	<i>Chamaeleo jacksonii</i>	28.9 × 16 (26–33 × 14–18) SI = 1.81 (1.53–2.14)	cylindrical	occasionally 1 absent	10.6 × 7.2 (9–12 × 6–8) SI = 1.47 (1.25–1.67)	gall bladder epithelium	Kenya	Modrý et al. 2000
<i>Eimeria hajeki</i>	<i>Rampholeon temporalis</i>	30.2 × 23.5 (29–31 × 22–25) SI = 1.3 (1.2–1.4)	broadly ellip- soidal	absent	10.8 × 8.8 (9–11.5 × 7.5–10)	unknown, oocysts in faeces	Tanzania	Modrý et al. 2001a
<i>Choleoimeria brookesiae</i> comb. n.	<i>Brookesia decaryi</i>	25.6 × 15 (23–27 × 13–16) SI = 1.71 (1.53–1.93)	cylindrical	absent absent	10.1 × 6.9 (9–11 × 6–7) SI = 1.45 (1.29–1.67)	unknown, oocysts in faeces	Madagascar	Modrý et al. 2001b
<i>Choleoimeria glawi</i> comb. n.	<i>Furcifer pardalis</i>	27.7 × 18.4 (26–29.5 × 17–19) SI = 1.51 (1.37–1.62)	cylindrical to ellipsoidal	absent absent	7.3 × 5.2 (6.5–8 × 5–5.5) SI = 1.4 (1.3–1.5)	gall bladder epithelium	Madagascar	Modrý et al. 2001b
<i>Eimeria vencesi</i>	<i>Furcifer pardalis</i>	14.3 × 13 (13–15.5 × 12–13) SI = 1.1 (1–1.25)	spherical to subspherical	usually 1–3 absent	7.3 × 5.2 (6.5–8 × 5–5.5) SI = 1.4 (1.3–1.5)	enterocytes of small intestine (nuclei)	Madagascar	Modrý et al. 2001b
<i>Eimeria worthi</i>	<i>Furcifer oustaleti</i>	17.9 × 15 (17.5–19.0) SI = 1.19	spherical	absent absent	8.2 × 5.8 (7–9.5 × 5–6.5) SI = 1.42	unknown, oocysts in faeces	Madagascar	Modrý et al. 2001b
<i>Choleoimeria hirbayah</i> sp. n.	<i>Chamaeleo calyptratus</i>	28.3 × 14.8 (25–30 × 13.5–17.5) SI = 1.92 (1.63–2.19)	cylindrical	absent absent	10.1 × 6.9 (9–11 × 6–7.5) SI = 1.48 (1.38–1.69)	gall bladder epithelium	unknown, found in captive animals	this study



**Fig. 9.** *Choleoeimeria hirbayah* sp. n.; composite line drawing of sporulated oocyst. Longitudinal suture dividing sporocyst wall in two plates is schematically shown on the upper sporocyst. Scale bar = 10  $\mu$ m.

**Type locality:** Species described from captive animals (F2 generation).

**Type material:** Photosyntypes are deposited under the collection No. R 8/03 in the Department of Parasitology, University of Veterinary and Pharmaceutical Sciences, Brno. Histological sections deposited under No. H 14/04 *ibid.*

**Site of infection:** Endogenous stages develop in the cytoplasm of the gall bladder epithelial cells.

**Etymology:** The specific name *hirbayah* is an Arabic term for chameleon; it is given as a noun in apposition.

## DISCUSSION

Eimerian coccidia represent a diverse assemblage of apicomplexan protists differing both in the oocyst morphology and the site and character of endogenous development. Two main lineages are evident within *Eimeria* s.l. in reptilian hosts based on a combination of sporocyst excystation structures and site and characters of the endogenous development: (i) species possessing Stieda body – *Eimeria* s.s., and, (ii) species with sporocyst wall composed of two plates joined by a meridional suture. In the latter group, three assemblages can be traced: species parasitizing biliary epithelium – *Choleoeimeria*, (ii) epicytoplasmic species with endogenous stages in gastrointestinal mucosa – *Acroeimeria* Paperna et Landsberg, 1989 and (iii) species with intracytoplasmic development in gastrointestinal mucosa. The

**Table 2.** Summary of experimental trials and results of infection of *Chamaeleo calytratus* with *Choleoeimeria hirbayah* sp. n.

Animal No.	Infection dose (oocysts)	First record of oocysts in faeces (DPI*)	Died (DPI)	Oocysts in gall bladder
1	60,000	57	138	yes
2	25,000	no	14	no
3	25,000	no	42	yes
4	25,000	no	51	yes
5	25,000	50	405	yes
6	25,000	83	575	yes

\*DPI – day post infection

separate status of the genus *Choleoeimeria* as a sister clade to the Eimeriidae was recently confirmed by a phylogenetic analysis of small subunit ribosomal RNA gene (Jirků et al. 2002).

Despite the fact that the family Chamaeleonidae comprises over 130 species in six genera (Nečas 2004), only 15 species of coccidia from the genera *Eimeria* (eight species) and *Isospora* (seven species) have been described to date (Table 2) (Sergent 1902, Modrý and Koudela 1995, Modrý et al. 1997, 2000, 2001a, b). Several eimerian coccidia of chameleons should be reclassified as *Choleoeimeria* based on data about oocyst morphology and endogenous development. Localisation of endogenous development in the gall bladder, elongated cylindrical oocysts and sutures in the sporocyst wall are the major distinguishing features. Based on these features, five eimerian species described originally as *Eimeria* should be referred to as *Choleoeimeria* (Table 1).

Considering morphological and developmental traits, the biliary coccidium described in this study is classified as another member of *Choleoeimeria*. *Choleoeimeria glawi* (Modrý, Daszak, Volf, Veselý, Ball et Koudela, 2001) comb. n., *C. brookesiae* (Modrý, Daszak, Volf, Veselý, Ball et Koudela, 2001) comb. n., *C. largeni* (Modrý, Šlapeta et Koudela, 2000) comb. n. and *C. bohemiai* (Modrý, Šlapeta et Koudela, 2000) comb. n. differ from *C. hirbayah* in oocyst size and/or oocyst shape or both. Oocysts of *C. glawi*, *C. brookesiae* and *C. bohemiai* are less elongated, and those of *C. largeni* are more elongated (Modrý et al. 2000, 2001a, b). *Choleoeimeria tilburyi* (Modrý, Šlapeta et Koudela, 2000) comb. n. from Kenyan *Chamaeleo jacksonii* possesses remarkably similar oocysts (Modrý et al. 2000). However, *C. hirbayah* can be distinguished based on the absence of polar granules in oocysts. Furthermore, oocysts of *C. hirbayah* differ from those of *Eimeria vencesi*, *E. worthi* and *E. hajeki* in oocyst size and shape (Modrý et al. 2001a, b).

*Choleoeimeria* oocysts are typically found in the faeces of their host in the fully sporulated stage (Paperna and Landsberg 1989, Lainson and Paperna 1999, Lainson 2003) and sporulation is classified as endoge-

nous (Paperna and Landsberg 1989). However, our experimental trials with *C. hirbayah* revealed different results. In initial phase of patent period in experimentally infected chameleons (DPI 50–60), all oocysts observed in faeces were unsporulated but the percentage of sporulated oocysts rose during patency. In animals shedding oocysts after ~4 months, >90% of oocysts seen were sporulated. In dissected animals originating from the colony a mixture of sporulated and unsporulated oocysts was present in their gall bladder contents.

Based on these facts, we assume that sporulation itself is a time-dependent process. In infected animals, most oocysts probably remain in the gall bladder content long enough to sporulate and in later stages of the infection the proportion of fully sporulated oocysts increases.

Importantly, we did not observe oocysts of *C. hirbayah* in intestinal contents in two of four dissected animals, despite the fact they had heavily infected gall bladders with a presence of oocyst mass in the lumen. This diagnostic failure can be caused by obturation of bile ducts by oocyst coagulum or tissue debris and might correspond with the observed dilatation of gall bladder. Such false negative results of faecal examina-

tion have consequences not only for the evaluation of data on the prevalence of biliary coccidia of reptiles, but also for clinical interpretations of coprological examinations in captive reptiles.

Coccidian parasites usually are considered as non or low pathogenic for their reptilian hosts (Levine 1988, Barnard and Upton 1994, Mader 1996, Greiner 2003). However, *Isospora jaracimrmani* is a significant pathogen in breeding colonies of veiled chameleons and *Isospora amphiboluri* McAllister, Upton, Jacobson et Kopit, 1995 was reported to be associated with mortality in captive bearded dragons, *Pogona vitticeps* Ahl, 1926 (McAllister et al. 1995, Modrý et Koudela 1998, Kim et al. 2002). Results of our experimental trials documented a high pathogenicity of *C. hirbayah* for juvenile and subadult chameleons. Actually, all experimentally infected animals died in various intervals after the inoculation (Table 2). In all cases, significant gall bladder dilatation was present during necropsy; clinical and pathological data will be published elsewhere.

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