ISOSPORA CORVI RAY, SHIVNANI, OOMMEN AND BHASKARAN, 1952 FROM THE COMMON HOUSE CROW (CORVUS SPLENDENS VIEILLOT) OF SELANGOR, PENINSULAR MALAYSIA

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Abstract. Faecal samples of 56 common house crows (Corvus splendens Vieillot) were collected from the Petaling Jaya and Kelang districts of Selangor, Peninsular Malaysia, and examined for coccidia. Intestinal tracts of 8 of the above crows were histologically examined under light microscopy to determine the site of coccidial infection and the endogenous stages present. Fifty three (94.6 %) crows had coccidial oocysts morphologically conforming to only one species of Isospora in their faeces at the time they were examined. The sporulated oocysts were found to be Isospora corvinae (Ray et al. 1952) which has been emended to I. corvi. These oocysts are redescribed in greater detail. Corvus splendens is a new host record for I. corvi. Coccidial infection was observed in all the intestinal tracts and generally confined to the anterior two thirds of the intestine. The parasites occurred within intestinal epithelial cells, located usually above the host cell nucleus. Developmental stages of both the asexual and sexual phases were found in the epithelium, and are deemed to be the endogenous stages of I. corvi on the basis of the oocysts recovered from the same crows used for histological study. These stages are described here for the first time. The prevalence of I. corvi, its relationship with the host C. splendens, and its probable transmission from C. macrorhynchos are discussed.

Coccidia rank among the most common parasites of birds. However there have been relatively few studies on the coccidia of passerine birds in nature. Of the 70 or so species of Isospora Schneider, 1881 reported from birds of the order Passeriformes, most occur in smaller passerines, e.g. canaries (Box 1977), house sparrows (Levine 1982a) and tree finches (McQuiston and Wilson 1988). Almost all were identified on the basis of oocyst morphology and very little is known about their endogenous stages. The genus Corvus, within the diverse and highly successful family Corvidae, contains many species of typical crows which are generally large passerine birds (Goodwin 1976). Yet it is surprising to note that only 5 species of Isospora have been reported from Corvus hosts (Pellérdy 1974) and none of these has been adequately described from the common house crow, Corvus splendens. In the course of an ecological study on the helminth parasites of this nuisance corvid from Selangor, Peninsular Malaysia (Kyi and Poon 1987), unsporulated coccidial oocysts were observed in the rectal contents of many birds. After sporulation, it was clear that they were oocysts of a species of Isospora. This prompted the present study, the main purpose of which was to identify and describe, with light microscopy, the oocyst and endogenous stages of the coccidian parasite that naturally infects house crows of this part of Malaysia. Its prevalence in the population of crow sampled, as well as its relationship with the host and its probable origin, are briefly discussed.

MATERIALS AND METHODS

Faecal samples were collected from 56 house crows killed in the field by controlled shooting. Shooting was carried out in the late afternoon to coincide with the return of the crows to their roosting sites. Faecal contents of each crow were stored in 2.0 % aqueous (W/V) potassium dichromate (K₂Cr₂O₇) solution. In the laboratory, the coarse faecal debris was removed by filtration through a 100 mesh brass sieve and the filtrates were thinly spread in petri dishes for 2—4 days at room
temperature to allow coccidial oocysts, if present, to sporulate. Oocyst suspensions were stored at 8°C until used. Oocysts for examination by light microscopy were concentrated on cover slips by zinc sulphate (33 % W/V ZnSO₄) solution. For histological study of the site of infection and the endogenous stages, intestinal tracts of 8 freshly shot birds were used. For each bird, short lengths of the duodenum, jejunum, ileum, rectum and cæcum were removed and fixed overnight in alcoholic Bouin's fluid. Faecal contents were also collected and treated as described earlier. Gut tissues were transferred to 70 % ethanol for storage and later processed for routine paraffin infiltration. Transverse sections, 6–8 μm thick, were cut and stained in Harris haematoxylin and eosin. Selected sections were stained with periodic acid – Schiff haematoxylin. Oocysts and histological slides were examined and photographed with a Dialux photomicroscope. Measurements of oocysts were taken from microscope projections made with a drawing tube. Endogenous stages in sections were measured with an ocular micrometer. All measurements, unless otherwise stated, were in microns (μm).

RESULTS

A total of 56 faecal samples from shot house crows was examined for coccidial oocysts. A high (94.6 %) prevalence of coccidiosis was recorded as 53 out of 56 crows were found to contain sporulated oocysts of only one species of Isospora. No oocysts of other coccidia were found. The same type of oocysts was also present in all the 8 crows used for histological study. Morphological characteristics of the exogenous stage were used to establish the identity of the isosporan species in question. All the birds were visibly healthy. No macroscopic lesions of the gut were observed at necropsy. Histological examination revealed the presence of various endogenous stages of the coccidial parasite in all the 8 intestinal tracts with no observable damage in the gut tissues. The posterior ileum and rectal region were not parasitized. The endogenous stages are considered to belong to the same species of Isospora as no other species were found in the faecal samples.

Isospora corvi Ray, Shivmani, Oommen et Bhaskaran, 1952

Description:

Exogenous stages. Sporulated oocysts (Fig. 1; Pl. I, Figs. 1–4) were spherical to subspherical, measuring 15.5–21.4 × 13.6–20.0 (mean 18.5 ± 0.10 × 17.12 ± 0.10, n = 135), with a length-width ratio of 1.00–1.25 (mean 1.08 ± 0.004), without a micropyle and polar granule and contained 1 or 2 small inconspicuous residual bodies. The oocyst wall was smooth, colourless to light brown and composed of a single layer about 0.3 to 0.4 thick. Sporocysts (Pl. I, Fig. 4) were elongate oval, measuring 11.8–16.8 × 6.8–10.5 (mean 14.3 ± 0.1 × 8.8 ± 0.1, n = 100), with a length-width ratio of 1.31–1.89 (mean 1.63 ± 0.01), and a distinct Stieda body and substiedal body at the more tapering end. The sporocyst residuum was relatively large, consisting of many small granules clumped together forming a compact ball. Sporozoites were elongate, somewhat banana-shaped, one end slightly broader and more rounded and lying lengthwise head to tail within the sporocyst. Accurate measurements of excysted sporozoites could not be obtained because of their extremely faint outlines. When some sporozoites emerged after in vitro excystation, they were found to be about 11.7–13.0 long and 2.5–3.3 wide. Each sporozoite contained a large refractile globule at the broad end. The sporulation time for the oocysts was 24–26 hours at 25 ± 2°C.

Comparative descriptions of oocysts of Isospora spp. from passerines belonging to the genus Corvus are given in Table 1.

Endogenous stages. The site of infection was the intestinal tract and the site of localization of the endogenous stages the intestinal epithelium. The parasites were
<table>
<thead>
<tr>
<th>Isospora spp.</th>
<th>Host</th>
<th>Oocyst</th>
<th>Sporocyst</th>
<th>Location of endogenous stages</th>
<th>Locality/country</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Isospora corni</em></td>
<td><em>Corvus splendens</em> Vieillot (house crow of Malaysia)</td>
<td>15.5 — 21.4 x 13.6 — 20.0 (18.5 x 17.1)</td>
<td>Spherical to subspherical</td>
<td>11.8 — 16.8 x 6.8 — 10.2 (14.3 x 8.8)</td>
<td>Elongate ovoid</td>
</tr>
<tr>
<td><em>Isospora corni</em></td>
<td><em>Corvus macrorhynchos intermedius</em> Wagler (common Himalayan crow)</td>
<td>15.0 — 23.0 x 14.0 — 21.5 (20.0 x 17.7)</td>
<td>Spherical to subspherical</td>
<td>7.5 — 12.5 x 6.25 — 8.75 (10.8 x 7.7)</td>
<td>Oval</td>
</tr>
<tr>
<td><em>Isospora bengalensis</em></td>
<td><em>Corvus splendens</em> Vieillot (house crow of India)</td>
<td>19.0 — 23.0 (range not given)</td>
<td>Round</td>
<td>15.4 x 7.7 (range not given)</td>
<td>Pyriform</td>
</tr>
<tr>
<td><em>Isospora monedulae</em></td>
<td><em>Corvus monedula collaris</em> (collared jackdaw)</td>
<td>Spherical: 16 — 20 (aver. 18) Oval: 16 — 22 x 14 — 18</td>
<td>Spherical to oval</td>
<td>12 — 16 x 6 — 8</td>
<td>Pyriform</td>
</tr>
<tr>
<td><em>Isospora rochaisi</em></td>
<td><em>Corvus sp.</em> (raven)</td>
<td>Round: 17 — 25 (aver. 22.9) Subspherical 19 — 27 x 25 (aver. 24.9 x 22.9)</td>
<td>Spherical to subspherical and oval</td>
<td>10.5 — 14.7 x 8.4 — 10.5</td>
<td>Pyriform</td>
</tr>
<tr>
<td><em>Isospora schwetzii</em></td>
<td><em>Corvus corone corone</em> (carrion crow)</td>
<td>Round to oval</td>
<td>Absent</td>
<td>Absent</td>
<td>No data</td>
</tr>
</tbody>
</table>

* Source of data from original authors  
** Source of data from Pellerdy (1974)
distributed mainly along the anterior two-thirds of the intestine, mostly occupying the villous epithelial cells. In one intestine which showed a heavy and extensive infection, the parasites had invaded the subepithelial tissues. The usual intracellular location of the parasites was the basal portion of the host epithelial cell above its nucleus but occasionally below it as well. Developing endogenous stages of the asexual and sexual phases were observed in all the crow intestines with a preponderance

Fig. 1. Composite line drawing of a sporulated oocyst of *Isospora corvi* from the faeces of the common house crow *Corvus splendens* from Selangor, Peninsular Malaysia.

of the gametocyte stages. Small numbers of almost spherical immature and mature schizonts (Pl. I, Figs. 5 and 6, Pl. II, Figs. 1 and 2) were observed in the villous epithelium lining the duodenum and anterior jejunal regions. Their size, based on 10 schizonts in sections, was 7.9 - 9.1 × 7.8 - 8.0 with a mean of 8.4 ± 0.1 × 8.2 ± 0.1. Up to 16 uninucleated cigar-shaped merozoites, arranged around a central cytoplasmic residuum, were seen in a fully developed schizont. Measurements, made from 16 merozoites that were in full longitudinal view, showed that they were 4.0 - 4.9 long and 0.9 - 1.6 wide with a mean of 4.5 ± 0.1 × 1.2 ± 0.1. Developing gametocytes (Pl. II, Figs. 3 - 6; Fig. 2 A - C) were the most common and conspicuous stages dispensed over the length of the intestine. They were spherical, ovoid to elongate and varied greatly in size according to their state of maturity and the plane of sectioning. Male gametocytes (microgametocytes) were not distinguishable from the female gametocytes (macrogametocytes) at the early immature stages. Mature microgametocytes contained numerous fully-differentiated, comma-shaped and intensely stained microgametes which were randomly arranged around a prominent cytoplasmic residuum (Pl. II, Fig. 5). Microgametocytes sometimes occurred in aggregates among the much more abundant macrogametocytes. Their dimensions, based on 50 gametocytes in sections, were 7.1 - 15.8 × 5.4 - 9.8 with a mean of 9.7 ± 0.2 × 7.5 ±
Macrogametocytes were observed at various stages of development (Fig. 2 A—C). More mature macrogametocytes, called macrogametes (Fig. 2 C) contained a characteristically central nucleus surrounded by peripheral, darkly-stained granules. Their dimensions, based on 50 gametes in sections, were 9.2—14.8 x 5.8—10.4 with a mean of 11.2 ± 0.2 x 8.0 ± 0.2. Zygotes undergoing wall formation, (Fig. 2 D) and mature oocysts with fully — developed walls, were often seen in close proximity.

Fig. 2. A—D Endogenous stages of *Isospora corei* in sections of crow intestine stained with haematoxylin and eosin (x 2000 except Fig. A) A — Group of macrogametocytes (arrow), each possessing a characteristic central nucleus and peripheral granules (x 800). B — Immature macrogametocyte (arrow). C — Mature macrogametocyte or macrogamete (arrow). D — Zygote (arrow) with fully formed oocyst wall. Note clearly visible parasitophorous vacuole (arrowhead).

Host epithelial cells harbouring the later stages of gametogony were clearly hypertrophied and strongly vacuolated (Fig. 2—D). The host cell nucleus, although occasionally displaced, showed no alteration in shape and size. There was no apparent host tissue response against the presence of parasites. Some oocysts freed in the intestinal lumen in sections appeared morphologically similar to unsporulated ones recovered from fresh faecal samples.

**DISCUSSION**

For many years species of *Isospora* from passerine birds have been identified primarily on the basis of oocyst structure and host species in which the oocysts were
found since biological characteristics were mostly unknown or poorly known. Our description of the isosporan species in question highlights the morphological characteristic of its exogenous and endogenous stages. As far as is known, only 5 species of *Isospora* identified solely on the basis of oocyst structure, have been reported from typical crows of the genus *Corvus: Isospora monedulae* from the collared jackdaw *Corvus monedula collaris* (Yakimoff and Matschoulsky 1936), *I. rodhaini* from the raven *Corvus* sp. (Yakimoff and Matschoulsky 1938), *I. schuetzi* from the carrion crow *C. corone corone* (Pelléryd 1974), *I. bengalensis* from the Indian house crow *C. splendens* (Mandal and Chakravarty 1964), and *I. corei* from the Indian Himalayan crow *C. macrorhynchos intermedium* (Ray et al. 1952). The original name *I. corei* has been emended to *I. corvi* in conformity with the rules of nomenclature and Latin grammar. The oocysts recovered from the local common house crow *Corvus splendens* are morphologically characteristic of *Isospora corvi*, first described in India by Ray et al. (1952) from *Corvus macrorhynchos intermedium*, a larger but very close relative of *C. splendens*. *C. splendens* is thus a new host record for *I. corvi*. Both host species are also common crows of India with a wide geographical distribution (Iamba 1976). Although oocysts of *I. corvi* from *C. splendens* in the present study (18.5 × 17.1) are slightly smaller than those from *C. macrorhynchos* (20.0 × 17.7) reported by Ray et al. (1952), there is an overlap in their sizes. This is also true of the larger sporocysts of *I. corvi* from *C. splendens* which are otherwise structurally similar with those from *C. macrorhynchos*. The subsisted body, first reported in this study, was previously overlooked by Ray et al. (1952). Their original description of the oocyst of *I. corvi* as being 'double contoured', probably in reference to its wall, is vague and misleading since their line drawings depict a wall delineated by two close lines. It appears that their term 'double contoured' is meant to denote the outer and inner boundaries of a single-layered oocyst wall. Our observations are that the oocyst wall of *I. corvi* is distinctly single-layered. Pelléryd (1974), in recapitulating the characteristics of *I. corvi* oocysts, made no reference to the original term. Of the other isosporan species infecting *Corvus* hosts, *Isospora rodhaini* and *I. schuetzi* differ from *I. corvi* by having distinctly larger oocysts which do not possess a cytoplasmic residuum. The oocyst of *I. bengalensis* is round, having a double layered wall and micropyle but no residuum, features which easily distinguish it from a *I. corvi* oocyst. *I. monedulae* differs from *I. corvi* mainly in the absence of oocystic residuum.

It is well established that eimerid coccidia typically parasitize the intestinal tracts of vertebrates (Levine 1982b). Histological studies of infected host intestines reveal that *I. corvi* is characterized by a number of distinct endogenous stages of the asexual and sexual phases all of which occur as intracellular parasites of the intestinal epithelium of the house crow *C. splendens*. Parasite distribution is restricted to the anterior two thirds of the intestine. Box (1977) distinguished *I. serini* and *I. canaria* from experimentally infected canaries on the basis of the locations and structural characteristics of their endogenous stages. The presence of various developing stages of schizonts, gametogony and oogony in anatomically close sites of the intestinal epithelium suggests that in nature *I. corvi* is able to complete its life cycle in the small intestine of house crows. We could not determine the generation of the schizonts observed nor be sure that the observed locations of schizonts represent all the asexual stages of the species. In *I. serini* Box (1977) found the asexual schizogonic generations took place not only in the intestinal epithelium of canaries but also in other extraintestinal sites such as the lung, liver and spleen. The preponderance of sexual gametocytic stages in infected crow intestines is very likely to be related to some particular phase of the parasite life cycle which has yet to be studied.

The high prevalence (94.6%) of *I. corvi* infection in the population of crows
sampled, the lack of pathological condition or disease and the ability to coexist intracellularly during its development suggest that this isosporan parasite in nature is easily transmitted and established in house crows, with which it seems to enjoy a harmonious and intimate relationship. *I. corvi* may have been transmitted to *Corvus splendens* from its relative *C. macrorhynchos* because of the close ecological association and overlapping range of these two corvid host species (Lamba 1976, Medway and Wells 1976, Goodwin 1976). The occurrence of *I. corvi* may have resulted from geographical proximity or from transmigration or transplantation of the host species. It is of interest to note that a number of Indian house crows from the Ceylon (now Sri Lanka) population were introduced near Kelang in the Selangor district of Peninsular Malaysia in 1903 to control insect pests of coffee plants (Wiley 1904). Since then, the Kelang house crow population has increased tremendously in Selangor (Siew et al. 1980), spreading and becoming a nuisance pest to human settlements along the west coast of Peninsular Malaysia (Medway and Wells 1976, Charles 1978).

Acknowledgements. We are grateful to Professor Emeritus Norman D. Levine of the University of Illinois for his most valuable advice on the taxonomy and name of *I. corvi*. Our thanks go to Viji and Lin Eng for their technical assistance, Che Rohani for typing the manuscript, and the University of Malaya for financial support.

REFERENCES


Received 10 October 1989
Accepted 31 August 1990
Fig. 1—4. Photomicrographs of the exogenous stages (oocysts) of *Isospora corvi* (×800 except Figs. 3). Fig. 1. Unsporulated oocysts. Fig. 2. Sporulated oocysts. Fig. 3. Sporulated oocyst (×2 000). Fig. 4. Some sporocysts freed from oocysts.

Figs. 5 and 6. Endogenous asexual stages of *I. corvi* in histological sections of crow intestinal villi stained with haematoxylin and eosin (×2 000). Fig. 5. Immature multinucleate schizont (arrow). Fig. 6. Maturing schizont with 16 transversely cut merozoites (arrow) around a central cytoplasmic residuum.
Fig. 1—6. Endogenous asexual and sexual stages of *Isospora corvi* in sections of crow intestinal villi stained with haematoxylin and eosin (×2,000). **Fig. 1.** Group of schizonts (arrow) and a gametocyte (arrowhead). **Fig. 2.** Mature schizont (arrow) with obliquely sectioned merozoites. **Fig. 3.** Immature microgametocyte (arrow) with many nuclei on the surface. **Fig. 4.** Microgametocyte (arrow) with peripheral nuclear material. **Fig. 5.** Mature microgametocyte with comma-shaped microgametes (arrow) around a cytoplasmic residual mass (arrow). **Fig. 6.** Mature microgametes (arrowhead) about to rupture out from parent gametocyte.