ON THREE COCCIDIAN PARASITES WENYONELLA MACKINNONI N.SP., EIMERIA LUCKNOWENSIS N.SP., AND ISOSPORA SP., FROM THE INTESTINE OF THE WAGTAIL MOTACILLA ALBA LINN. (PASSERIFORMES, MOTACILLIDÆ)

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Received on December 17, 1946
(Communicated by Dr. G. D. Bhalerao)

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INTRODUCTION

During the winter of 1940, eight specimens of the common wagtail Motacilla alba Linn. were entrapped in Lucknow and an examination of their droppings revealed a coccidial infection in two out of eight birds. In order to study the exogenous stages of development of this coccidian, the droppings as well as the rectal contents of the infected birds after dissection were kept in 1 per cent. solution of chromic acid. Each oocyst, after sporulation, showed four sporocysts inside it, and each sporocyst in turn had four sporozoites, i.e., four sporocysts and sixteen sporozoites were present in each oocyst—a diagnostic character of the genus Wenyonella Hoare, 1933.

During the winter of 1941, six more specimens of the same bird were examined for coccidial infection; out of these five proved to be coccidia-free, but one was passing two kinds of oocysts along with its faeces: (i) oval oocysts, which were colourless, and (ii) spherical oocysts with thick yellowish inner cyst walls. In 1 per cent. solution of chromic acid, after complete sporulation, these oocysts were diagnosed as belonging to the genera Eimeria Schneider, 1875, and Isospora Schneider, 1881, respectively.
The coccidiosis-free specimens of *Motacilla alba* could not be infected artificially, as they died, (probably they could not stand confinement for long), before the oocysts of the three above-mentioned coccidian parasites could sporulate and attain the infective stage in the culture medium.

Pieces of small intestine were fixed in Bouin-Duboscq-Brazil, sectioned 4–6 μ thick, and stained with iron-alum haematoxylin and chromotrop 2 R, or Delafeld's haematoxylin only. A few fresh smears of the scrapings of the intestine were made in normal saline solution and examined under an oil-immersion lens, but no motile stages of the parasites could be detected. Similar smears fixed in Schaudinn's fluid and stained with iron-haematoxylin were also examined but they did not yield any significant result besides those that had been obtained from a study of the sections of the intestine.

It may be mentioned here that only six species of *Wenyonella* have been recorded up to date (*vide* Table I). However, the species of *Wenyonella* described in this paper differs from those mentioned above in certain particulars, and therefore, I propose to designate this coccidian of the wagtail as *Wenyonella mackinnoni* n.sp., the specific name being given in honour of Prof. Doris L. Mackinnon of the King's College, London.

*Wenyonella mackinnoni* n.sp.

**Exogenous stages.**—The oocysts are spherical or ovoid in shape; they measure 19-23 μ in spherical forms, and 23.8 μ–26.2 μ × 18.0 μ–21.5 μ in ovoid forms. The cyst wall consists of two layers: an outer layer which is thin and colourless, and an inner layer which is comparatively thicker and is brownish in colour. The protoplasm of the freshly discharged oocyst is filled with refractile granules of reserve materials and occupies the entire internal space (*Text-Fig. I, 1*), but later on it becomes condensed and has a more or less spherical contour and measures, on an average, 15.5 μ, in diameter (*Text-Fig. I, 2*); the micropyle and the polar inclusions are absent in the oocysts.

Oocysts kept in 1 per cent. solution of chromic acid and examined at regular intervals of six hours at room temperature revealed visible signs of segmentation of the zygotes within 24 to 36 hours, and within next 48 to 60 hours four rounded bodies, the sporozoites, were cleaved out of the protoplasmic bulk of the zygote. Usually the sporoblasts remain adhering together for some time, but later on they separate (*Text-Fig. I, 3*), become ovoid, and each of them secretes a wall around itself and thus forms the sporocyst (*Text-Fig. I, 4*). There is no oocystic residue left after the formation of the sporocysts. Each sporocyst measures 10.2 μ × 7.4 μ in
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size, and has a lens-shaped thickening at its narrower end. The protoplasm of the sporocyst in turn segments, without leaving any residue, into four rounded bodies, the precursors of the sporozoites, which later on elongate (8.2 μ long) and assume a club-shaped appearance (Text-Fig. 1

(All figures were drawn with the aid of camera lucida.)

Text-Fig. 1.—Showing exogenous stages of Wenyonella mackinnoni n. sp.
From living specimens × 1900.

1, a freshly discharged oocyst. 2, oocyst with unsegmented but condensed zygote. 3, oocyst showing formation of sporoblasts. 4, oocyst with four sporocysts. 5, 6, oocysts showing sporocysts, each with four sporozoites.

5, 6). They are arranged at random inside the sporocysts. The formation of the sporozoites takes place during the next 24 to 48 hours after the formation of the sporocysts, i.e., complete sporulation takes 4 to 6 days.
Endogenous stages.—The endogenous cycle of development takes place in the small intestine of the host. It may be mentioned at once that no asexual or schizogonic stages of the parasite could be detected, and the only stages frequently encountered were: (i) the microgametocytes and microgametes, (ii) the macrogametocytes and macrogametes, and (iii) the zygotes. On one occasion only a few microgametes were noticed within the cavity inside an epithelial cell lodging a macrogamete (Text-Fig. II, 11); but no stage showing the entrance of a microgamete into the macrogamete could be encountered. Besides these stages, young developmental stages of the sexual forms, measuring 2.2 µ-6.0 µ, were also seen on certain occasions (Text-Fig. II, 1-5): they have been designated as the sexual forms, following Ray and Das Gupta's (1937) view of distinguishing the schizonts from the sexual forms of *W. hoarei* and also because they exhibit resemblances to the mature sexual forms in their cytoplasmic and nuclear contents.

The entire absence of schizogonic cycle may be due to the fact that it was over when the birds were examined. It seems that this parasite, like other coccidia, also undergoes a course of "self-limited" infection.

The grown-up microgametocytes (Text-Fig. II, 6) measure, on an average, 20.5 µ x 15.6 µ in size and can be distinguished from the macrogametocytes, besides their size, by having (i) a conical shape, (ii) a ragged cytoplasm, and (iii) a centrally located nucleus with a centrally situated karyosome which is comparatively smaller than that of the macrogametocyte. A mature microgametocyte gives rise to several microgametes (Text-Fig. II, 7), leaving a considerable bulk of cytoplasm unused. Each microgamete (Text-Fig. II, 8) has an elongated body (3.8 µ long) and two equal flagella which are nearly twice the length of the body. Whether the flagella are attached anteriorly or posteriorly is difficult to say, because the movements of the microgametes could not be observed in vivo.

The grown-up macrogametocytes (Text-Fig. II, 9) are ellipsoidal bodies, rounded at both ends, and measure, on an average, 28.5 µ x 16.0 µ in size. The cytoplasm of each macrogamocyte contains reserve materials, and the nucleus lies rather nearer the superior pole. The karyosome is fairly big and excentric in position, being surrounded by a clear space. During the course of its development the macrogamocyte becomes more or less globular in shape, and gives rise to a single macrogamete (Text-Fig. II, 10, 11) measuring 23.0 µ x 19.8 µ in size. On no occasion could a micropyle be detected in the macrogametes of *Weynonella mackinnoni* (cf. *W. hoarei* Ray and Das Gupta, which possesses a prominent micropyle.
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The zygotes (Text-Fig. II, 12) can be distinguished from the macrogametocytes and macrogametes by having (i) a denser accumulation of reserve materials (the so-called plastic and haematoxylinophilic granules), and (ii) a homogeneously stained nucleus.

**Text-Fig. II.**—Showing endogenous stages of *Wenyonella mackinnoni* n. sp.

From sections of small intestine.

Figs. 1–5. Developing sexual forms × 1750. 1, 2, microgametocytes. 3, 4, 5, macrogametocytes; in fig. 3 two parasites are seen in a single cell. Figs. 6–12. Mature sexual forms. 6, a microgametocyte × 1000. 7, showing several microgametes and a central “restkörper” (semi-diagrammatic) × 1000. 8, a highly magnified microgamete × 2500. 9, a macrogametocyte × 1050. 10, 11, macrogametes; in fig. 11 a few microgametes are seen lying near the macrogamete × 1050. 12, a zygote × 1050.

**Diagnosis.**—Tetrazoic tetrasporocystid condition of the oocysts determines the position of this coccidian under the genus *Wenyonella* Hoare, 1933.

Oocysts spherical or ovoid, measuring 19·0 μ–26·2 μ × 18·0 μ–21·5 μ; cyst wall thick, double-layered, outer colourless, inner brownish; micropyle absent; sporocysts ovoid, measuring 10·2 μ × 7·4 μ, with a lens-shaped


<table>
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<tr>
<th>Name</th>
<th>Oocysts</th>
<th>Sporulation period</th>
<th>Sporocysts</th>
<th>Host</th>
<th>Habitat</th>
<th>Locality</th>
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<tr>
<td></td>
<td>Shape</td>
<td>Measurements</td>
<td>Residue</td>
<td>Shape</td>
<td>Measurements</td>
<td>Residue</td>
</tr>
<tr>
<td>1 W. africana</td>
<td>Subspherical or ovoid</td>
<td>18.5—19.2 X</td>
<td>Absent</td>
<td>5—7 days</td>
<td>Ovoid, lensiform knob present at the narrower pole</td>
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<td></td>
<td></td>
<td>16.0—17.6</td>
<td></td>
<td></td>
<td>Do</td>
<td></td>
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<tr>
<td>2 W. hoarei</td>
<td>Spherical</td>
<td>14.9—18.5</td>
<td>Do</td>
<td>7</td>
<td>Ovoid, both ends similar, lensiform knob absent</td>
<td>Do</td>
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<tr>
<td>Ray and Das Gupta, 1935</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3 W. uelensis</td>
<td>Ovoid</td>
<td>26.0—30.0 X</td>
<td>Transient</td>
<td>5</td>
<td>Ovoid, both ends similar, lensiform knob absent</td>
<td>Do</td>
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<tr>
<td>Berghe, 1938</td>
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<td>19.0—20.0</td>
<td></td>
<td></td>
<td>Do</td>
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<tr>
<td>4 W. paria</td>
<td>Subspherical</td>
<td>15.2—13.3</td>
<td>Absent</td>
<td>7</td>
<td></td>
<td></td>
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<td>Berghe, 1938</td>
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<tr>
<td>5 W. bahli</td>
<td>Subspherical or ovoid</td>
<td>16.0—17.5 X</td>
<td>Do</td>
<td>4—5</td>
<td>Egg-shaped lensiform knob absent</td>
<td>Absent</td>
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<tr>
<td>Misra, 1944</td>
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<td>14—6—15.5</td>
<td></td>
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<tr>
<td>6 W. gallinae</td>
<td>Oval or egg-shaped</td>
<td>29.48—33.60 X</td>
<td>?</td>
<td>4—6</td>
<td>Short necked round-bottomed vials</td>
<td>Present</td>
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<tr>
<td>Ray, 1945</td>
<td></td>
<td>19.84—22.78</td>
<td></td>
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<td></td>
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<tr>
<td>7 W. machinnoni n. sp.</td>
<td>Spherical or ovoid</td>
<td>19.0—26.2 X</td>
<td>Absent</td>
<td>Do</td>
<td>Ovoid, lensiform knob present at narrower pole</td>
<td>Absent</td>
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<tr>
<td></td>
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<td>18.0—21.5</td>
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knob at one end; sporozoites 8.2 μ long, club-shaped, irregularly arranged; oocystic and sporocystic residuum bodies absent; sporulation time 4 to 6 days; unsegmented oocysts discharged in the faeces of the host.

**Systematic position.**—*Wenyonella mackinnoni* n.sp. (Eimeriidae, Coccidiidae).

**Habitat.**—Small intestine of *Motacilla alba* Linn.

**Locality.**—Lucknow, U.P., India.

The accompanying table shows a comparison of the known species of *Wenyonella* with regard to oocysts, sporocysts, hosts, etc.

**Eimeria lucknowensis** n.sp.

To the best knowledge of the author, there is only one species of *Eimeria*, namely, *E. roscoviensis* (Labbé, 1893)* recorded from *Motacilla alba* Linn. This parasite has, however, been reported from other birds as well, e.g., *Phalacrocorax aristotelis*, *Charadrius cantianus*, *Strepsilas interpres*, *Nummius phaeopus*, *Pulvialis apricarius*, *Totanus calidris*, etc. (**vide** Levine and Becker, 1933). Labbé in his previous paper (1893) did not give any illustration of the oocysts of *E. roscoviensis*, but in a later contribution (1896) he supplemented a figure (**vide** his Pl. XVII, fig. 18) of the mature oocyst of this coccidian, which gives a clear idea of the structure of its oocyst. A comparison of the mature oocyst of the species of *Eimeria* described in this paper with that of *E. roscoviensis*, as sketched by Labbé, would at once reveal that the latter species does not coincide in its characters with the former, and therefore, a new specific name, *Eimeria lucknowensis* n.sp., has been instituted for the present coccidian.

The oocysts of *E. lucknowensis* are ovoid in shape and are eliminated in an unsegmented condition in the faeces; they measure 21.4 μ-24.5 μ × 17.4 μ-18.8 μ in size. The cyst wall is colourless and double-layered; both ends of the oocysts are similar and rounded, and there is no indication of a flattening or prolongation at either end, nor is there any evidence of a micropyle and polar inclusions. In these characters *E. lucknowensis* differs markedly from *E. roscoviensis*, in which the oocysts are pyriform in shape, measure 16.0 μ-18.0 μ × 14.0 μ-16.0 μ, and each mature oocyst is characterized by the presence of a truncated neck bearing a pseudomicroyle, as well as the polar globules. In 1 per cent. solution of chromic acid the oocysts of *E. lucknowensis* sporulate within 3 to 4 days. There is no residual body inside the oocyst after the formation of the sporozoites.

* Labbé in his original paper (1893, p. 408) has named it as *Coccidium roscoviense*. 
which are four in number, and which later on secrete a wall around each one of them, thus giving rise to the same number of sporocysts (Text-Fig. III, 1–3). Each sporocyst is ovoid in shape, and is devoid of any thickening at either pole; it measures $8.5\mu \times 6.0\mu$. The end-product of sporogony is the formation of two club-shaped, curved sporozoites within each sporocyst; the sporozoites measure $7.0\mu$ in length and are arranged with their concavities facing the sporocystic residuum between them. The sporocyst of *E. roscoviensis*, on the other hand, is pyriform in shape, and has a knob-like thickening at its narrower pole; moreover, the two sporozoites in each sporocyst lie on one side, the other side of the sporocyst being occupied by the residual body.

A study of the endogenous stages found in the small intestine was not conclusive, because of the simultaneous presence of the endogenous stages of another coccidium *Isospora* sp. described below.
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Diagnosis.—D'zoie tetrasporocystid condition of the oocysts places this coccidian under the genus Eimeria Schneider, 1875.

Oocysts ovoid, 21·4 μ-24·5 μ × 17·4 μ-18·8 μ, discharged unsegmented in the faeces; sporocysts ovoid, 8·5 μ × 6·0 μ; sporocystic residue in between the two sporozoites; sporulation period 3 to 4 days.

Systematic position.—Eimeria lucknowensis n.sp. (Eimeriidae, Coccidiidae).

Habitat.—Small intestine of Motacilla alba Linn.

Locality.—Lucknow, U.P., India.

Isospora sp.

Only one coccidian belonging to the genus Isospora, namely, I. passerum* Sjöbring, 1897, has been reported from Motacilla alba Linn. This parasite, however, has been recognised as a synonym of Isospora lacazei (= Diplospora lacazii) Labbé, 1893, of the passerine birds, and held by certain workers to be a pathogenic species. Thus Labbé (1893) mentioned that I. lacazei proved fatal to faeces infected experimentally with sufficient doses of this parasite. Hadley (1910) asserted that the common English sparrow and other birds, if chanced to find access into the poultry runs, could transmit† white diarrhoea to young fowls and blackhead to turkeys, the causative agent being the same parasite. Becker (1934) stated that this "parasite has a special interest because it is a cause of loss among caged birds, particularly canaries".

Existence of more than one species of Isospora in passerine birds has been suggested by several protozoologists, e.g., Labbé (1893), Wenyon (1926), Becker (1934), etc., but cross-infection experiments have not been conducted to support their views. Labbé (1893), however, recorded Isospora rivolta (= Diplospora rivolta) from chaffinch, speckled magpie and titmouse (all passerines), the distinguishing characters of this coccidian being the comparatively heavier wall of its oocysts, and the oocysts required not less than 15 days (Labbé in 1896 mentions "douze a quinze jours") for development, whereas in I. lacazei the walls of the oocysts are thinner, and the oocysts required 4 to 5 days (Labbé in 1896 mentions "trois ou quatre jours") for sporulation. Although Labbé has given no illustrations of I. rivolta, the above-mentioned differences, as well as the differences in the measurements of the oocysts (in I. lacazei 23 μ-25 μ and in I. rivolta 16 μ-18 μ), are,

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* Also known as Isospora communis-passerum Sjöbring, 1897.

† Hadley's announcement of the pathogenicity of this parasite has, from cross-infection experiments, been proved untenable by Smith and Smillie (1917), Johnson (1923), Boughton (1929), etc., since all attempts to infect fowls with this coccidian have met with failure.
I think, quite suggestive of regarding these two parasites as distinct and separate species.* Becker (1934, p. 101) has also expressed that the species of *I. rivolta*, "as well as some new ones, may have to be recognised". However, the present species of *Isospora* differs in certain respects from *I. lacazei*, but in the size of its oocysts, comparatively thicker cyst walls, and delayed period of sporulation, it approximates to *I. rivolta*, and therefore, it has been avoided to dub a new specific name to it. The distinguishing characters of this coccidian are given below.

The oocysts are spherical, 14.8 μ-17.8 μ in diameter, and are discharged in an unsegmented condition along with the faeces of the host; the cyst-wall is two-layered, the inner layer being comparatively thick and yellowish in colour, while the outer one is thin and colourless; micropyle and polar inclusions are absent; sporulation (Text-Fig. III, 4-6) in 1 per cent. solution of chromic acid requires 10 to 12 days; two sporocysts are formed in each oocyst and the oocystic residue is absent. Each sporocyst, measuring 10.6 μ × 7.4 μ, is ovoid in shape having one pole rounded and the other narrower, the latter having a nipple-like knob at its extremity; the Steida body is invariably absent in the sporocyst. The contents of the sporocysts undergo segmentation and thus four spindle-shaped sporozoites measuring 7.5 μ in length are formed, and a voluminous residue is left inside each sporocyst. The arrangement of the sporozoites does not follow any regular order.

Endogenous stages were not conclusive due to a mixed infection (*vide supra*).

**Diagnosis.**—Tetrazoic sporocystid condition of the oocysts locates this coccidian under the genus *Isospora* Schneider, 1881.

Oocysts spherical, 14.8 μ-17.8 μ, unsegmented in fresh faeces; sporocysts ovoid, with nipple-like knob, 10.6 μ × 7.4 μ; sporulation time 10 to 12 days.

**Systematic position.**—*Isospora* sp. (Eimeriidae, Coccidiidae).

**Habitat.**—Small intestine of *Motacilla alba* Linn.

**Locality.**—Lucknow, U.P., India.

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* The coccidian *I. rivolta* Grassi, 1879, which inhabits the intestines of cats and dogs, has been mentioned as *I. rivolta* by certain writers, e.g., Leuckart (1886, p. 221, *Coccidium rivolta*), Dobell and O'Connor (1921, p. 98), etc. If *I. rivolta* (Labbé, 1893) is recognised as a valid species, it is suggested, in order to avoid confusion between the two different parasites —the one occurring in cats and dogs and the other in birds, that the name *I. rivolta* should be substituted by *I. labbei*, n. n.
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ACKNOWLEDGMENTS

The author takes this opportunity to express his sincere thanks of gratitude to Prof. K. N. Ball, of the Lucknow University, for supervising this work; to Dr. H. N. Ray, Protozoologist at the Imperial Veterinary Research Institute, Miktesar, for confirming the observations; and to Dr. B. N. Chopra, Offg. Director, Zoological Survey of India, for giving facilities to consult the necessary literature.

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