EIMERIA SAITAMAЕ N. SP.: A NEW CAUSE OF COCCIDIOSIS IN DOMESTIC DUCKS (ANAS PLATYRHYNCHA VAR. DOMESTICA)

Isamu Inoue
Saitama Veterinary Experiment Station, Bessho,
Urawa City, Saitama Prefecture

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According to Becker8), duck coccidiosis was first reported by JüRN. It has been reported from many countries. There have been no papers published in Japan on the occurrence of anseriform coccidiosis. In the autumn of 1964, the author encountered an outbreak of duck coccidiosis in Saitama Prefecture, Japan.

The author and his associates8-11) mentioned the results of clinical investigation and the life cycle of the coccidium which had been isolated in the form of oocyst from infected ducks. Having compared this oocyst with that of the known anseriform Eimeria organism, they presumed that the isolated oocyst might be of new species.

The present paper deals with the results of identification of the coccidium isolated in the 1964 outbreak and the description of a new species called Eimeria saitamaе by the author.

EXPERIMENTAL DESCRIPTION

Oocysts (Figs. 1 to 2)
Each of the unsporulated oocysts isolated was almost completely filled with a zygote. Its wall had a double layer and a smooth surface, being 0.7-0.8 μ in thickness. There was a distinct micropyle at its narrower end, especially when it was a sporulated specimen. Having been cultured in 2 percent potassium bichromate suspension, an unsporulated oocyst was placed in a petridish and allowed to stand at 25°. It began to be divided into sporonts at 12 hours of cultivation, and 4 sporocysts were formed at 24 hours. Furthermore, the suspension became clear at 48 hours. A polar inclusion was also observed. At 60 hours was formed a residual body. The sporulation was finished at 72 hours.

The sporulated oocyst was ovoid and colorless, and had a smooth wall, containing 4 sporocysts. Each sporocyst had 2 sporozoites in it. Fifty sporulated oocysts measured 17 to 21 (18.62±1.44) by 13 to 15 (13.21±0.47) μ.

Schizogony and gametogony (Figs. 3 to 16)
Eighteen 10 to 14-day-old Peking ducklings (Anas platyrhyncha) were administered directly into the crop with a half million to one million sporulated oocysts and placed in wirefloored cages.

Specimens were collected from them and stained with Heidenhain iron-hematoxylin. Free sporozoites, which had been released from the oocyst in 3 hours after the infection, measured 8 μ in length and 1.5 μ in width. In 12 hours they had invaded the epithelial cells of the intestines, becoming round in shape. Young first-generation schizonts were seen 24 hours after the infection. Twelve hours later, first-generation schizonts were almost completely formed and contained large merozoites.

Forty-eight hours after the appearance of first-generation schizonts other types of schizonts were recognized. They had small merozoites of radiant form in them and round debris in their center. At this time a number of merozoites appeared, looking like so many bananas. At 60 hours, it was possible to find two different types of schizonts and two forms of merozoites, large banana-shaped and small-sized. The large merozoites were 6.2 to 10.0 (8.3±0.23) by 1.5 to 3.1 (2.2±0.09) μ in size, and the small merozoites 3.5 to 5.6 (4.5±0.18) by 1.2 to 1.8 (1.4±0.06) μ. Second-generation schizonts also began to appear. Macro- and microgametocytes were seen at 72 and 96 hours. The former presented a very characteristic appearance. The latter were of spindle or willow-leaf shape and 5 to 9 by 0.5 to 10 μ in size.

Prepatent period

Twelve 10-day-old Peking ducks (Anas platyrhyncha) were infected with 20,000 sporulated oocysts. Fecal samples were collected and subjected to microscopic examination following centrifugal flotation in Sheather's sugar solution. In every case, oocysts were detected on the 4th day and for the following three days.

Cross infection

Five 22-day-old chickens were infected with 130,000 sporulated oocysts. They were examined for twelve days after application of Sheather's method. No oocysts nor clinical symptoms could be recognized.

Two 15-day-old goslings (African geese) were infected with 26,000 sporulated oocysts and examined by the same method as mentioned above. No infection, however, was successfully established. This result indicates that these oocysts were not parasitic for chickens and goslings.

Location (Figs. 17 and 18)

Smear specimens of the intestinal contents were stained with Heidenhain iron-hematoxylin, fixed in 10 percent formalin solution, embedded in paraffin, and stained with hematoxylin and eosin (H-E) and periodic acid-Schiff (PAS) stain. Microscopic examination revealed that the parasite infected a portion extending from the duodenum to the cecum, and that the infection was the severest in the middle or posterior portion of the small intestine.

In sections, it was demonstrated that schizonts and gametocytes grew mostly in the epithelial-cell layer and occasionally in the lamina propria mucosae.

DESCRIPTION of THE SPECIES

Twenty species of Eimeria have been described among those of the avian order Anseriformes.

Eimeria truncata RAILLIET and LUCET, 1890(17) was reported from the domestic goose (Anser anser anser). Its oocyst is 22 to 20 by 16 to 13 μ in size. The parasite invades the epithelium of the uniniferous tubules and takes such shape as different by host. It has been reported from the domestic goose by a number of investigators.

Eimeria anseris KOTŁAN, 1932(19) emend. KOTŁAN, 1933(19) has been reported from the domestic goose (Anser anser anser). Its oocyst measures 13 to 18 by 16 to 23 μ, being colorless and piriform. Its prepatent period was seven days and its sporulation time 1 to 2 days.

Eimeria nocens KOTŁAN, 1933(19) has been reported from the domestic goose (Anser anser anser). Its oocyst, which measures 17 to 24 by 25 to 33 μ, is ovoid or ellipsoid in shape, flat at the micropylar end, and is provided with a brownish outer wall.

Eimeria sp. TIBOLDY, 1933(19) has been reported from the domestic duck (Anas...
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*platyrhynchus domesticus*. Its oocyst measures 10.8 to 25.0 by 8.0 to 12.6 μ and is oval, elongate oval, or occasionally round. It is possible that several species are involved in what has been reported as a single species.

*Eimeria parvula* KOTLÁN, 1933(2) has been reported from the domestic goose (*Anser anser anser*). Its oocyst, which measures 10 to 14 by 10 to 15 μ, is round or ellipsoidal, and has no micropyle.

*Tyzzeria perniciosa* ALLEN, 1936(1) has been reported from the domestic Peking duck (*Anas domestica*). Its oocyst measures 9 to 10.8 by 10 to 13.3 μ, is round, and has no micropyle. There are no sporocysts. Eight banana-shaped sporozoites are formed indirectly.

*Eimeria bucephala* CHRISTIANSEN and MADSEN, 1948(5) has been reported from the golden-eye duck (*Bucephala clangula clangula*). Its oocyst measures 13 to 20 by 25 to 39 μ and is ovoid or ellipsoidal, and light brown.

*Eimeria magnalabra* LEVINE, 1952(13) has been harbored by blue and lesser snow geese (*Anser coeruleans*) and a Canadian goose (*Branta canadensis interior*). Its oocyst is 15.1 to 17.3 by 21.7 to 24.0 μ in size and slightly ovoid, and has a brownish-yellow wall and no polar inclusion. The micropyle is prominent and surrounded by thick lips which hang down into the interior of the oocyst.

*Eimeria somateriae* CHRISTIANSEN, 1952(13) has been reported from the long-tailed duck (*Clangula hyemalis*) and common eider (*Somateria mollissima mollissima*). It measures 10.6 to 19.2 by 21.2 to 41.3 μ. Its oocyst is commonly elliptical, with one of its poles drawn out like the neck of a bottle, where there is a micropyle. Schizogony and gametogony occur in the kidney.

*Eimeria striata* FARR, 1953(6) has been reported from the eastern Canada goose (*Branta canadensis canadensis*). Experimentally, the species was proved to be transmissible to the domestic goose (*Anser anser anser*). Its oocyst measures 13.7 to 18.0 by 18.9 to 23.6 μ, is elliptical or ovoid, and has a pale-yellow outer wall, a prominent micropyle, and one or more polar granules.

*Eimeria hermani* FARR, 1952(6) has been reported from the eastern Canada goose (*Branta canadensis canadensis*). Its oocyst measures 17.5 to 19.5 by 24.3 to 27.6 μ, is ovoid, and has a prominent micropyle and no polar inclusion.

*Eimeria fulva* FARR, 1953(6) has been reported from the eastern Canada goose (*Branta canadensis canadensis*). Experimentally, the parasite can be made to persist in the domestic goose. Its oocyst measures 20.2 to 25.2 by 25.6 to 32.4 μ, is broadly ovoid and occasionally almost piriform, and has hanging lips and a brownish-yellow wall. The prepatent period is nine days.

*Eimeria brantae* LEVINE, 1953(6) has been reported from the lesser Canada goose (*Branta canadensis parva*). Its oocyst measures 17.7 by 23.4 μ, is ovoid, and has thick lips around the micropyle.

*Eimeria anatis* SCHOLTYSECK, 1955(8) has been reported from the mallard (*Anas platyrhyncha platyrhyncha*). Its oocyst measures 10.8 to 15.6 by 14.4 to 19.2 μ, and is ovoid. The micropyle seems to be closed by a plug-like body. One sporozoite of the sporocyst is larger than the other sporozoite.

*Eimeria clarki* HANSON, LEVINE and IVENS, 1957(9) has been reported from the blue and lesser snow goose (*Anser coeruleans coeruleans*). Its oocyst measures 18 to 21 by 25 to 30 μ, looking like a round-bottomed flask, and has a prominent micropyle at the protruding anterior end.

*Eimeria farri* HANSON, LEVINE and IVENS, 1957(9) has been reported from the white-fronted goose (*Anser albifrons frontalis*). Its oocyst measures 17 to 20 by 22 to 23 μ, is
ellipsoidal or slightly ovoid, and colorless or very pale yellow, and has no micropyle.

_Eimeria boschidis_ WALDEN, 1961 has been reported from the mallard (Anas platyrhyncha platyrhyncha). Its oocyst measures 11.8 to 13.1 by 18.3 to 26.5 µ and is bottle-shaped. A micropyle is present.

_Eimeria battakhi_ DUBEY, 1963 has been reported from the domestic duck (Anas platyrhynchos domestica). Its oocyst measures 16 to 21 by 19 to 24 µ and is nearly sub-spherical or somewhat ovoid. The outer wall is pale yellow to orange and the inner one dark green. A micropyle is absent.

_Eimeria stigmosa_ KLIMEŠ, 1963 has been reported from the domestic goose (Anser anser anser). Its oocyst measures 23.0 by 16.7 µ, on the average, and has a dark brown wall. A micropyle is present.

_Eimeria danailovi_ GRÄFNER, 1965 has been reported from the domestic duck. Its oocyst measures 11.44 to 14.56 by 18.72 to 22.88 µ and is ovoid. The wall is yellowish-green or green. There are two corpuscles on the opposite side of the micropyle.

**DISCUSSION**

The oocyst of _Eimeria saitamae_ n. sp. can be distinguished from that of _Tyzzeria perniciosa_ by its small, round body containing no sporocysts. The oocyst of _Eimeria truncata_ is distinguished by its infection in the kidney. The oocyst of _Eimeria anseris_ is distinct by its characteristic morphology, viz., piriform. The oocyst of _Eimeria nocens_ is distinguished by its relatively large size and brownish outer wall. The oocyst of _Eimeria paruila_ is round and small in size and has no micropyle. The oocyst of _Eimeria bucephalae_ is distinguished by its light-brown wall. The oocyst of _Eimeria magnalabia_ has a brownish-yellow wall and hanging lips, but no polar inclusion. The oocyst of _Eimeria somateriae_ is distinguishable, since it is harbored in the kidney. The oocyst of _Eimeria hermani_ is distinguished by the absence of polar inclusion and prominent micropyle. The oocyst of _Eimeria striata_ has a pale yellow outer wall, a prominent micropyle, and one or more polar inclusions. The oocyst of _Eimeria fulva_ is distinguishable by its typical morphology, viz., broadly ovoid or piriform, and the possession of hanging lips and a brownish-yellow wall. The oocyst of _Eimeria brantae_ is noted for the presence of thick lips. The oocyst of _Eimeria anatis_ is distinguished by its distinct morphology, having prominences like horns on the outer layer in the part where a micropyle is found. The oocyst of _Eimeria farii_ has a very pale yellow wall, but has no micropyle. The oocyst of _Eimeria boschidis_ is of bottle shape. The oocyst of _Eimeria clarkei_ is distinguished by its shape of round-bottom flask and its prominent micropyle. The oocyst of _Eimeria battakhi_ has no micropyle, and its outer wall is pale yellow to orange, and its inner one dark green. The oocyst of _Eimeria stigmosa_ is distinguished by its dark-brown wall. The oocyst of _Eimeria danailovi_ has a yellowish-green or green wall and two corpuscles. The oocysts of _Eimeria_ sp. that were reported by TIBOLDY involve those of several species which cannot be differentiated from one another. It is considered that they have no diagnostic value.

On the other hand, there is a difference in host species between the oocyst of _Eimeria saitamae_ and that of any other species that has ever been reported. _Tyzzeria perniciosa_, _Eimeria truncata_, _Eimeria anseris_, _Eimeria magnalabia_, _Eimeria nocens_, _Eimeria paruila_, _Eimeria brantae_, _Eimeria fulva_, _Eimeria clarkei_, _Eimeria farii_, _Eimeria hermani_, _Eimeria stigmosa_, _Eimeria striata_, and _Eimeria danailovi_ infect the domestic goose or are transmissible to this bird.

_Eimeria bucephalae_, _Eimeria battakhi_, _Eimeria anatis_, _Eimeria boschidis_, and _Eimeria somateriae_ are clearly distinguishable by their morphology as mentioned above.

The _Eimeria_ species newly described in the present paper differs from any of the
other species of the same genus that have ever been reported. Hence the new specific name *Eimeria satamae*.

**SUMMARY**

Coccidiosis broke out among ducklings in Japan in September, 1964. The following findings were obtained.

1) Oocysts were isolated from the feces of birds involved. They were colorless, ovoid, and 17 to 21 (18.62±1.44) by 13 to 15 (13.21±0.47) μ in size, having a micropyle. In them, the prepatent period was the 4th day and the sporulation time at 3 days.

2) The life cycle was studied, by using isolated oocysts. Free sporozoites were found in 3 hours after infection. In 12 hours they invaded the epithelial cell. Juvenile and mature schizonts were recognized 24 and 36 hours, respectively, after infection. Other types of schizonts appeared at 48 hours. Among them, merozoites were of radiant form with round debris at the center. Second-generation schizogony took place at 60 hours. Macrogameteocytes were seen at 72 and 96 hours, respectively.

3) The portions of the intestine where the isolated parasite had been harbored extended from the duodenum to the cecum. Severe infection was found in the middle and posterior parts of the small intestine.

4) The isolated oocysts were not infectious to chickens and goslings in the experiment.

5) Compared with the oocyst of every known species of the genus *Eimeria*, the isolated oocysts were considered as those of a new species. Accordingly, the author called the new species *Eimeria satamae*.

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**REFERENCES**

あひるコクシジウムの新病原体 *Eimeria saitamae*

井 上 勇

埼玉県家畜衛生試験場

(昭和42年3月28日受付)

1964年の9月に、埼玉県幸手町にあるあひる飼育場で、あひるのコクシジウム症に遭遇した。

1) 分離オーシストは、無色、卵円形で、micropyleを有し、大きさは17〜21（m.18.62±1.44）×15〜15（m.13.21±0.47）μである。25℃、3日の間で胞子の形成が認められた。また、再生日数は4日であった。

2) 人工感染を行ない、Heidenhain ironhematoxylin染色を施して発育環の観察を行なった。3時間後には、放出されるsporozoiteが、また12時間後には、宿主細胞に侵入した像が観られた。24時間後には、幼虫のschizontが、36時間後には、ほぼ完成された卵円形で、中に弓状・大型のmerozoiteを含んでいるschizontが認められた。48時間後には、残体を中心に、小型のmerozoiteが放射状に分布している。このことからschizontが出現した。60時間後には、第2代のschizontが認められ、72時間後にはmacrogametocyteが、96時間後にはmicrogametocyteが、それぞれ観察された。

3) 寄生部位は、十二指腸より盲腸におよんでいた。ここに小腸遊離部の中央部より下部にかけて、濃厚感染していた。また、大部分は粘膜上皮細胞に寄生していたが、一部は粘膜固有層にも認められた。

4) 鳥鳥と鶏の飼に人工感染を試みたが、感染力は有していなかった。

5) 分離オーシストを既知のオーシストと比較したが、いずれにも属さない新しい種類であった。そこで"Eimeria saitamae"と命名した。
EXPLANATION OF PLATES

Figs. 3 to 16 show sections stained with Heidenhain iron-hematoxylin. Fig. 17 shows a section stained with PAS and hematoxylin. Fig. 18 shows a section stained with hematoxylin and eosin.

PLATE I

Fig. 1. Unsporulated oocyst. ×1,000.
Fig. 2. Sporulated oocyst. ×1,000.
Fig. 3. Free sporozoite released from the oocyst in 3 hours after infection. ×1,200.
Fig. 4. Sporozoite invading an epithelial cell in 12 hours after infection. ×1,200.
Fig. 5. Juvenile schizont 24 hours after infection. ×1,200.
Fig. 6. Mature schizont 36 hours after infection. ×1,200.

PLATE II

Fig. 7. Juvenile schizont of radiant form 48 hours after infection. ×1,200.
Fig. 8. Mature schizont of radiant form 48 hours after infection. ×1,200.
Fig. 9. Free merozoite, large and banana-shaped, released from an oval schizont 48 hours after infection. ×1,200.
Fig. 10. Free merozoite released from a schizont of radiant form 60 hours after infection. ×1,200.
Fig. 11. Second-generation schizont 60 hours after infection. ×1,200.
Fig. 12. Young macrogametocyte 72 hours after infection. ×1,200.

PLATE III

Fig. 13. Mature macrogametocyte 72 hours after infection. ×1,200.
Fig. 14. Young microgametocyte 96 hours after infection. ×1,200.
Fig. 15. Young microgametocyte (left), mature microgametocyte, and microgamete 96 hours after infection. ×1,200.
Fig. 16. Mature microgametocyte with debris at the center 96 hours after infection. ×1,200.
Fig. 17. A number of young schizonts, in the epithelial cell in section. ×400.
Fig. 18. Mature schizont in the epithelial cell in section. ×400.