Tyzzeria perniciosa gen. et sp. nov., a coccidium from the small intestine of the Pekin Duck, *Anas domesticus* L.

By

Ena A. Allen,

Associate Zoologist, Zoological Division, Bureau of Animal Industry, U. S. Department of Agriculture, Washington D. C., U.S.A.

With plate 9—10.

In the small intestine of a young Pekin duck, received from Long Island on August 27, 1935, there was found a coccidium of a new genus and a new species of the family Eimeriidae, sub-family Cryptosporidiinae. The sporulated oöcyst resembles those of the genera *Pfeifferinella* and *Schellakia*, in being asporocystid, and in having 8 sporozoites lying directly within the oöcyst wall. The differential characteristics of the genera of the sub-family Cryptosporidiinae are given in the following key which is adapted from HOARE, 1933.

Key to the genera of the sub-family Cryptosporidiinae.

1. Oöcysts giving rise directly to 4 sporozoites . . . *Cryptosporidium*
   Oöcysts giving rise directly to 8 or more sporozoites 2
2. Oöcysts giving rise directly to many sporozoites . . *Lankesterella*
   Oöcysts giving rise directly to 8 sporozoites 3
3. Macrogametocyte with fertilization tube . . . . . *Pfeifferinella*
   Macrogametocyte without fertilization tube 4
4. Haemogregarine; life cycle in 2 hosts . . . . . *Schellakia*
   Not a haemogregarine; life cycle in 1 host . . . . . *Tyzzeria*

Tyzzeria n. gen.

Generic diagnosis. — Cryptosporidiinae: Sporulated oöcyst with 8 sporozoites, not having a sporocyst wall but lying free within the thick oöcyst wall; residual mass large, granular; macrogametocyte without a fertilization tube; entire life cycle in one host with exception of sporulation of oöcyst which takes place outside of host's body.

It is proposed to name this new genus *Tyzzeria* in honor of Dr. Edward Ernest Tyzzer, of the Harvard Medical School, Harvard University, who has contributed largely to our knowledge of the parasitic protozoa of birds, especially of the coccidia of gallinaceous birds.

Type species. — *Tyzzeria perniciosa* n. sp.

Tyzzeria perniciosa n. sp.

Specific diagnosis. — *Tyzzeria*: Oöcysts 9 to 10.8 μ by 10 to 13.3 μ, elliptical. Oöcyst wall thick, colorless, consisting of 2 layers, the outer thinner than the inner and very transparent, consequently not easily seen; no operculum or visible micropyle. Protoplasm of non-sporulated oöcyst coarsely granular, completely filling the space enclosed by the wall (Pl. 9 Fig. 1). Sporozoites 8 in number (Pl. 9 Figs. 2—4), developing directly within the wall of the oöcyst, there being no formation of a sporocyst. Sporozoites curved, one end rounder and broader than the other, about 10 μ long by 3.5 μ wide at the larger end. Residual mass large, composed of granules of various sizes, usually spherical (Pl. 9 Fig. 4), except in oöcysts kept for some time after sporulation in which case the granules of the mass sometimes becoming somewhat dispersed (Pl. 9 Fig. 3).

Host. — *Anas domesticus* L. (Pekin duck).

Location. — Small intestine.

Locality. — Rinebeck, Long Island, New York, U.S.A.

Type specimens. — No. 29,569, U. S. National Museum (Bureau of Animal Industry) Helminthological Collection.

Life cycle.

Two ducks about 6 weeks old which, on fecal examination, were negative for coccidia were fed sporulated oöcysts of *T. perniciosa* that were obtained from the Long Island duckling. Both birds

developed coccidiosis and the oöcysts proved to be *T. perniciosa.* Sporulation of oöcysts required about 24 hours. In order to study the developmental phases of this parasite, 15 Pekin ducks 1 to 3 weeks old were fed sporulated oöcysts of *T. perniciosa* and 1 duck was killed each day, on successive days. Various portions of the small intestine were fixed in BOUIN'S solution, sectioned and stained, some with iron-haematoxylin and some by MAXIMOW'S method.

The mucosa and sub-mucosa of the intestine, from the gizzard to the ceca, were invaded by this coccidium. In $5^1/_2$ hours from the time of inoculation, sporozoites, liberated from ingested oöcysts, had entered tissue cells of the intestinal wall and had rounded up; some had begun to divide (Pl. 9 Fig. 5). In heavy infections 2 or 3 sporozoites were found in 1 cell. At the end of 24 hours schizonts of the first generation (Pl. 9 Fig. 6) had developed; these were very small, about 8.3 by 11.6 μ, having only a few merozoites as compared with those formed later. By the fifth day following inoculation (Pl. 9 Fig. 7), schizonts completely filled many of the tissue cells. On the ninth day after inoculation schizonts appeared as shown in Pl. 9 Fig. 8, the pycnotic nucleus having been pushed to one side and attached to a small remnant of cytoplasm of the original tissue cell. At least 3, if not more, generations of schizonts developed, since schizogony continued long after the formation of gametocytes. The largest schizonts measured about 14 to 15 μ by 15 to 16 μ, being nearly spherical.

Gametocytes began their development very early, the first microgametocytes appearing 48 hours after inoculation. The microgametocytes measured about 5.8 by 7.5 μ; they were composed of a central core to which the tiny microgametes were fastened with their heads extending outward like those of pins in a pincushion (Pl. 10 Fig. 9). The developing macrogametocytes were somewhat irregular in shape (Pl. 10 Fig. 11), varying in size from about the size of a tissue cell nucleus to slightly larger bodies; in some of these, 1, 2 (Pl. 10 Fig. 10), or many chromatin-like bodies were noted, included in the protoplasm. The fact that the chromatin-like bodies or nuclei (?) varied considerably in size was thought to indicate that there may have been a number of nuclear divisions and that all except one nucleus, and the excess cytoplasm, may have become absorbed into the body of the developing gamete or zygote or, on the other hand, that there may have been more than one generation of gametocytes. More study of the sexual phase of the life-cycle of this organism is needed in order to interpret correctly, if possible, the development of the

zygote. The first oöcysts completed development in the tissue by the end of the fifth day following inoculation, and were first observed in the droppings on the sixth day.

Pathogenicity.

Symptoms. — Noticeable symptoms of ducks, infected with *T. perniciosa*, were loss of appetite, loss of weight, weakness manifested by the inability of the bird to stand for any length of time, and continuous crying as if in distress. This last symptom was especially noticeable in baby ducks.

Mortality. — Seven ducks 1 week old were each fed approximately $^1/_{10}$ cc. of a thoroughly mixed suspension of numerous oöcysts of *T. perniciosa* in water; 5 were kept for controls. On that same day 3 ducks, 7 weeks old, were given about 1 cc. of the same inoculum; 2 of this lot were kept as controls. Of the inoculated birds, 4 of the younger birds died on the afternoon of the fifth day; during that night the remaining 3 of the younger group and 1 of the older ducks also died. A very heavy infection developed in the second older duck; the third of this lot had only a light infection, probably due to its having lost most of the inoculum at the time of inoculation.

Macroscopic lesions. — *T. perniciosa* produced inflammation and hemorrhagic areas throughout the small intestine, especially in the upper half where the infection was usually heaviest; it also produced rounded white spots on the exterior of the intestinal wall and a thickening of the wall. In severe cases, the lumen of the small intestine was filled with blood and often contained a cheesy exudate; the latter, however, was not in the form of a core, as in the case of *Eimeria tenella* infections. The epithelial layer of the mucosa sloughed off in long pieces, sometimes separating as an inner tube which could easily be lifted out at necropsy. These lesions resembled closely those caused by *E. necatrix* of chickens.

Microscopic lesions. — Microscopic examination of tissue sections revealed that *T. perniciosa* penetrated both the mucosa and sub-mucosa of the intestinal wall to the muscle layers; this is a deeper invasion of the tissues than has been observed for any other coccidium of domestic birds. In very heavy infections, there was great destruction of tissue, for there was scarcely a cell that had not been invaded (Pl. 10 Fig. 12). In one severe case there were portions of the intestinal wall of which the muscular layers were perforated. This perforation, however, may have been due to the action of bacteria which entered as secondary invaders.

Comparison of the Genera *Pfeifferinella* and *Tyzzeria*.

The 2 species of the genus *Pfeifferinella*, known at the present time, are liver parasites; *P. ellipsoides* WASIELEWSKI, 1904, is a parasite of the fresh-water mollusc (*Limax cornua*), and *P. impudica* LÉGER and HOLLANDE, 1912, occurs in the land snail (*Limax marginatus*). *Tyzzeria perniciosa* is an intestinal parasite of the Pekin duck (*Anas domesticus*). Thus *Pfeifferinella* has an invertebrate host; that for *Tyzzeria* is a vertebrate. According to LÉGER and HOLLANDE, the mature gametocyte of *Pfeifferinella* has a vaginal or fertilization tube, a characteristic not found in *Tyzzeria*. The merozoites of *Pfeifferinella* appear like flagellates of the crithidia type, unlike those of *Tyzzeria* which are crescent-shaped. There are at least 3 types of schizonts in the life cycle of *Tyzzeria*, while only 1 type has been described for *Pfeifferinella*. Because of these differences, it is considered justifiable to place this coccidium of the duck in a new genus.

Other coccidia of the duck.

BECKER (1934) gives a historical account of coccidia reported in the duck by different authors, some of whom found clinical cases identified as infections with *Eimeria avium* and *E. tenella*. No critical studies of the specific identify of the coccidia were made. Attempts by JOHNSON (1923) to inoculate 4-month-old ducks with *E. tenella* were unsuccessful. TIBOLDY (1933) found that *Eimeria* from ducks, geese and chickens were strictly host specific. She found oöcysts of coccidia, in the duck, that were oval, elongate oval, or rounded, with a heavy wall; they measured 10 to 25 μ by 8 to 12.6 μ. No specific name was given to these coccidia. It would seem from these data that *T. perniciosa* is the first species of coccidia to be described from the duck.

Summary.

Tyzzeria perniciosa is described as a new genus and a new species of coccidia from ducks, with a key to the genera of the sub-family Cryptosporidiinae; also an account is given of its life-cycle.

Since this coccidium is very minute, it could easily be overlooked. It is highly pathogenic and, therefore, it is likely to be economically important.

Literature cited.

Becker, Elery R. (1934): Coccidia and coccidiosis of domestic, game, and laboratory animals and of man. 147 pp. Collegiate Press, Inc., Ames, Iowa.

Hoare, Cecil A. (1933): Studies on some new ophidian and avian coccidia from Uganda, with a revision of the classification of the Eimeriidae. Parasitology Vol. 25 p. 359—388.

Johnson, W. T. (1923): Avian coccidiosis. Poultry Science Vol. 2 p. 146—163.

Léger, L. et Hollande, A. Ch. (1912): La reproduction sexuée chez les coccidies Monosporées du genre Pfeifferinella. Archives du Zoologie Experimentale et Générale 5e Série T. 9 p. 1—8.

Tiboldy, Béla (1933): Kísérletes vizsgálatok a szárnyas baromfi coccidiumainak fajlagosságáról. Közlem. az Összehas. Élet-és Kórtan Köréből Vol. 26 p. 173—182. Budapest. (Extract from thesis.)

Wasielewski, Th. v. (1904): Studien und Mikrophotogramme zur Kenntnis der pathogenen Protozoen. p. 104—107. Leipzig.

Explanation of plates.

Plates 9—10.

Tyzzeria perniciosa n. gen. et n. sp.

Figures 1—11, inclusive, drawn with the aid of a camera lucida; magnification about 2900. Figure 12, a photomicrograph with magnification of 1520.

Plate 9.

Fig. 1. Non-sporulated oöcyst with the remains of a tissue cell attached.

Fig. 2. Sporulated oöcyst, side view, residual mass compact.

Fig. 3. Sporulated oöcyst, side view, residual mass somewhat dispersed.

Fig. 4. Sporulated oöcyst, end view.

Fig. 5. Sporozoites, rounded up in tissue cells; from the duodenum of a duck that had been inoculated $5^1/_2$ hours previously. One sporozoite with a dividing nucleus.

Fig. 6. First generation schizont from small intestine of duck, 24 hours after inoculation.

Fig. 7. Schizont, fifth day following inoculation.

Fig. 8. Schizont, ninth day following inoculation.

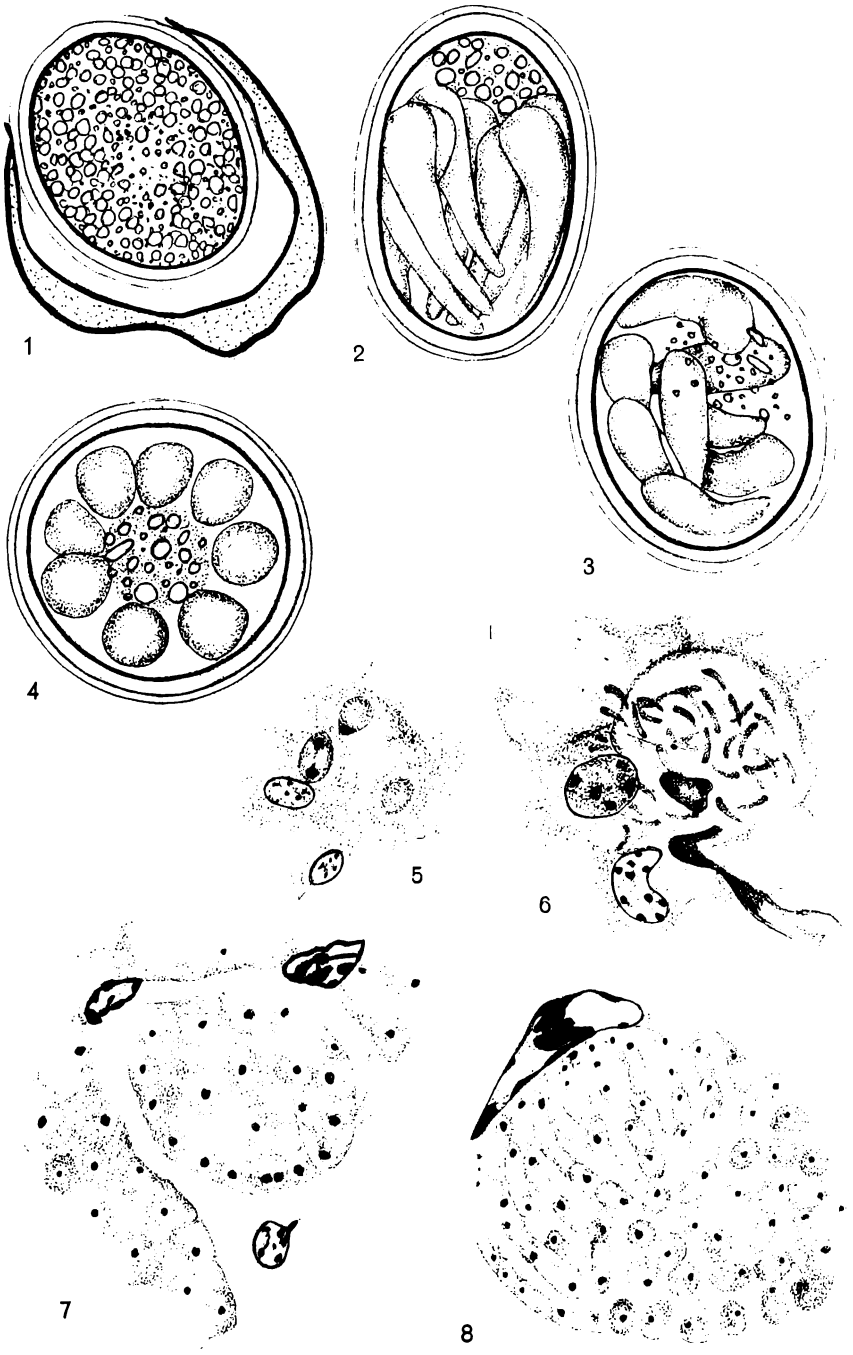
Plate 10.

Fig. 9. Microgametocyte.

Fig. 10. Tissue from small intestine of infected duck; *a*, a young macrogametocyte with 2 nuclei; *b*, a mature macrogametocyte; *c*, nucleus of tissue cell.

Fig. 11. Tissue from small intestine of infected duck; *d*, developing macrogametocytes having many chromatin-like bodies or nuclei (?); *e*, developing macrogametocyte having fewer chromatin-like bodies; *f*, nucleus of tissue cell, given for comparison of its size with that of macrogametocytes; *g*, another macrogametocyte, similar to *b* in figure 10.

Fig. 12. Photomicrograph of section of tissue, 2 μ thick, from duodenum of duck which died on fifth day following inoculation. Note complete destruction of tissue; arrows point to some of the schizonts that have invaded the tissue.



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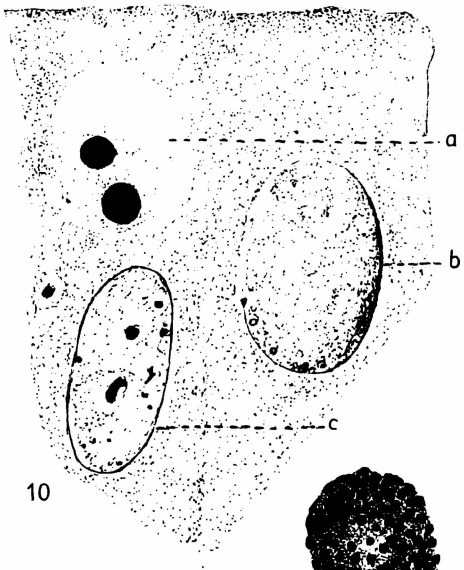
VERLAG VON GUSTAV FISCHER IN JENA



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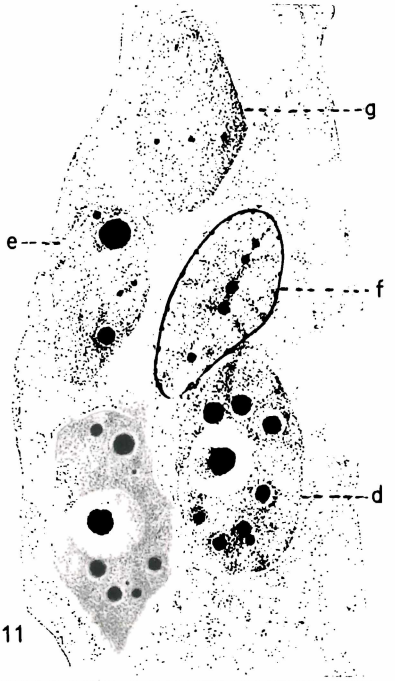

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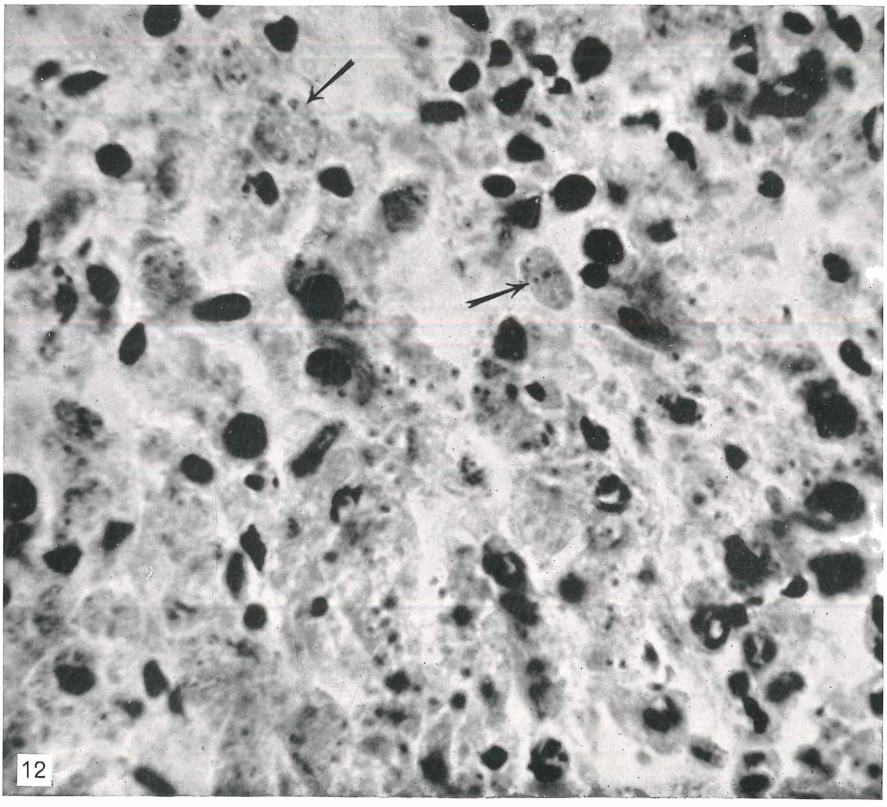

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VERLAG VON GUSTAV FISCHER IN JENA.