

ニホンカモシカから分離したコクシジウムの4新種

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Eimeria capricornis* n.sp., *E. nihonis* n.sp., *E. naganoensis* n.sp., and *E. kamoshika* n.sp. (Protozoa: Eimeriidae) from the Japanese Serow, *Capricornis crispus

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ABSTRACT. *Eimeria capricornis* n.sp., *E. nihonis* n.sp., *E. naganoensis* n.sp. and *E. kamoshika* n.sp. were detected from the Japanese serow, *Capricornis crispus*. The oocysts of these species were ovoid to ellipsoid, measuring $48.02 \pm 0.53 \times 33.93 \pm 0.35 \mu\text{m}$, $29.73 \pm 0.45 \times 21.01 \pm 0.29 \mu\text{m}$, $20.04 \pm 0.05 \times 15.77 \pm 0.04 \mu\text{m}$ and $30.02 \pm 4.24 \times 14.58 \pm 2.03 \mu\text{m}$, respectively. In all the species the micropyle was observed, but the micropylar cap and oocyst residuum were not present. The polar granules were observed in the oocysts of *E. capricornis*. The sporocysts of the above species were spherical to elongate ovoid, measuring $15-23 \times 8-13 \mu\text{m}$, $14-16 \times 7-10 \mu\text{m}$, $11-13 \times 6-8 \mu\text{m}$ and $8-10 \times 6-7 \mu\text{m}$, and the sporulation time was 6, 3, 3 and 6 days, respectively. The sporocyst residuum and refractile body were seen in all the species. In the sporocysts of *E. naganoensis* and *E. kamoshika* tiny Stieda body was seen, but not in the other two species. These four *Eimeria* species were not infective to goats.—**KEY WORDS:** coccidium, Japanese serow, morphology.

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Coccidia of the wild animals belonging to Caprinae have been reported from the tribes Saigini and Rupicaprini [1,2,4-12]. But there were no records on the coccidian species of the Japanese serow. The author compared the isolated coccidian oocysts from the Japanese serows with those of the known *Eimeria* species from the mammals closely related to *Capricornis*. As a result, it was found that the morphological features of the isolated four species were different from those of the other *Eimeria* species. The present paper deals with the morphology of the new *Eimeria* species, *Eimeria capricornis*, *E. nihonis*, *E. naganoensis* and *E. kamoshika*.

MATERIALS AND METHODS

Sample collection from the Japanese serows: during the period from December 1982 to March 1983 and from November 1983 to March 1984, a large number of

Japanese serows, *Capricornis crispus*, were caught in Nagano Prefecture. Of them, 214 carcasses were brought to Dr. A. Tsukise of the Department of Veterinary Anatomy, Nihon University, several days after being captured, and all the samples received by the author were rectal feces.

Fecal examination: Each fecal sample was mixed with 2% potassium dichromate solution and a thin layer of the fecal solution was placed in a Petri dish to sporulate oocysts at 25°C. The oocysts were concentrated by Sheather's flotation method with sugar solution. One hundred oocysts of each species were measured.

Inoculation to goats: Two 2-month-old domestic goats were individually inoculated with a mixture of 1,300 sporulated oocysts of *E. capricornis*, 1,100 of *E. nihonis*, 1,600 of *E. naganoensis* and 1,100 of *E. kamoshika*. The feces of the goats were examined for oocysts by Sheather's flotation method with sugar solution for 30 days after inocula-

tion.

RESULTS

Description of species

Eimeria capricornis n.sp.

Oocyst ovoid or ellipsoid, dark brown, with distinct micropyle (Fig. 1). 48.02 ± 0.53 by 33.93 ± 0.35 μm ($40.6-53.3 \times 30.5-40.6$ μm) in size, length-width ratio 1.42 (1.33 to 1.62) on average. Oocyst wall rough, about 3.0 ± 0.26 μm thick, composed of 3 layers. Outer layer dark brown, transversely striated, and easy to be separated from the middle layer. Middle layer pale yellow, inner layer violet. A polar granule present (Fig. 2), but micropylar cap oocyst residuum absent. Sporocysts elongate ovoid, 15 to 23 by 8 to 13 μm , sporocyst residuum present, but Stieda body absent. Large and small refractile bodies present in each sporozoite. Sporulation time 6 days.

Oocysts of *E. capricornis* were found abundantly in 80 (37.4%) of the 214 fecal samples.

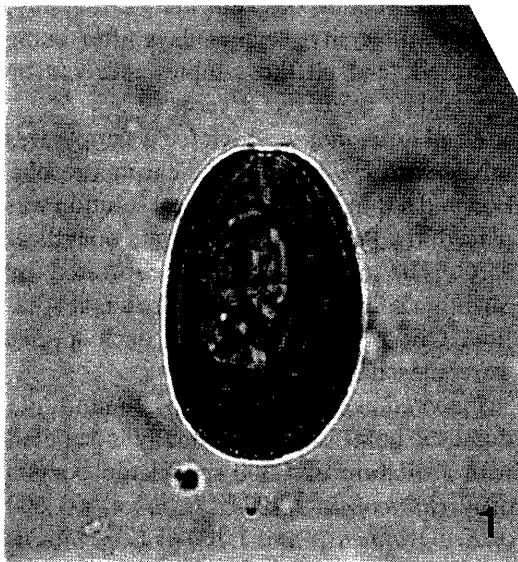


Fig. 1. *Eimeria capricornis*. Sporulated oocyst with dark brown rough wall and distinct micropyle. $\times 200$.

Eimeria nihonis n.sp.

Oocyst ellipsoid, pale brown, with a micropyle. 29.73 ± 0.45 by 21.01 ± 0.29 μm ($27.9-33.0 \times 19.1-22.9$ μm) in size, length-width ratio 1.42 (1.22 to 1.53) on average. Oocyst wall rough, about 1.7 ± 0.19 μm thick, composed of 2 layers; outer layer brown and transversely striated, inner layer black (Fig. 3). Polar granule, micropylar cap and oocyst residuum absent. Sporocysts spherical or short oval, 14 to 16 by 7 to 10 μm . Sporocyst residuum present, but Stieda body absent. Large and small refractile bodies present in each sporozoite. Sporulation time 3 days.

Oocysts of *E. nihonis* were found in 75 (35.1%) of the 214 samples.

Eimeria naganoensis n.sp.

Oocyst ovoid and pale brown, with a micropyle which is observed clearly when the oocysts were allowed to stand overnight (Fig. 5). 20.04 ± 0.05 by 15.77 ± 0.04 μm ($16.51-22.86 \times 13.97-17.78$ μm) in size, length-width ratio 1.27 (1.08 to 1.50) on average. Oocyst wall smooth, about 1.0 to

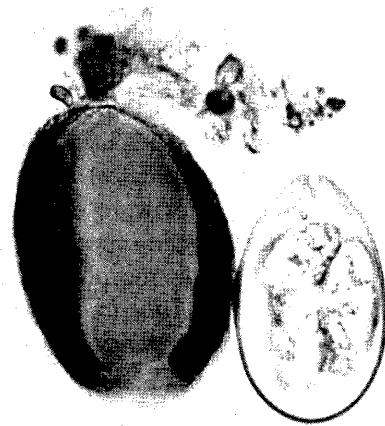


Fig. 2. *Eimeria capricornis*. Sporulated oocyst with removed outer layer of oocyst wall. Distinct polar granule is recognized. $\times 200$.

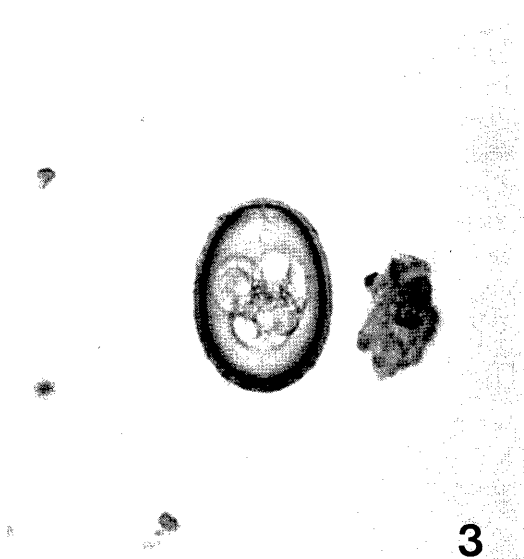


Fig. 3. *Eimeria nihonis*. Sporulated oocyst with brown rough wall. $\times 200$.

1.2 μm thick, composed of 2 layers; outer layer pale yellow and indistinctly transversely striated, inner layer violet (Fig. 4). Polar granule, micropylar cap and oocyst residuum absent. Sporocysts elongate ovoid, 11 to 13 by 6 to 8 μm . Sporocyst residuum and Stieda body present, but tiny and large refractile bodies present in each sporozoite. Sporulation time 3 days.

Oocysts of *E. naganoensis* were found in 25 (11.7%) of the 214 samples.

Eimeria kamoshika n.sp.

Oocyst elongate ellipsoidal, colorless, with a distinct micropyle. External shape asymmetrical, slightly flattened at the one side and convex at the opposite side. 30.02 ± 4.23 by 14.58 ± 2.03 μm (25.4 – 33.02×11.43 – 16.51 μm) in size, length-width ratio 2.06 (1.76 to 2.7) on average. Oocyst wall smooth, about 0.5 to 0.8 μm thick, composed of 2 layers; outer layer pale yellow, inner layer violet (Fig. 6). Polar granule, micropylar cap and oocyst residuum absent. Sporocysts spherical or short oval, 8 to 10 by 6 to 7 μm . Sporocysts spherical or short oval, 8 to 10 by 6 to 7 μm .

Sporocyst residuum and Stieda body present. Tiny and large refractile bodies present in each sporozoite. Sporulation time 6 days.

This species was found in only 4 (1.9%) of the 214 samples.

Infectivity to goats: Domestic goats inoculated with the respective oocysts of *E. capricornis*, *E. nihonis*, *E. naganoensis* and *E. kamoshika* passed no oocysts and developed no clinical symptoms.

DISCUSSION

As the *Eimeria* species detected from the related species to the Japanese serow, *Eimeria sajanica* and *E. saiga* have been reported from the saiga, *Saiga tatarica* [4, 10], *E. rupicaprae*, *E. riedmuelleri*, *E. yakimoffmatschoulskyi*, *E. alpina* and *E. supereri* from the chamois, *Rupicapra rupicapra* [1,2,9,12], and *E. oreamni*, *E. montanaensis* and *E. ernsti* from the Rocky Mountain goat, *Oreamnos americanus* [8,11].

The four new *Eimeria* species from the Japanese serow could be discriminated from all the above species by the morphology of oocysts, sporulation time and host specificity.

The oocyst of *Eimeria sajanica* Machul'skiĭ, 1947 [4] is distinguishable by its ovoid or spherical shape and size (20 by 18 μm), and that of *E. saiga* Svanbaev, 1958 [10] by its spherical shape and size (29.5 by 30.5 μm) and by the presence of a polar granule and an oocyst residuum. The oocyst of *E. rupicaprae* Galli-Valerio, 1924 [1] is ovoid and 18 to 32.5 by 13 to 27 μm in size. The outer layer of oocyst wall is yellowish and has a small micropyle. The sporulation time is 4 to 6 days. This species is similar to *E. naganoensis*, but differs from it in the shape of micropyle and sporulation time. The oocyst of *E. riedmuelleri* Yakimoff and Matschoulsky, 1940 [12] is spherical or ovoid and 15 to 23 by 14 to 22 μm in size,

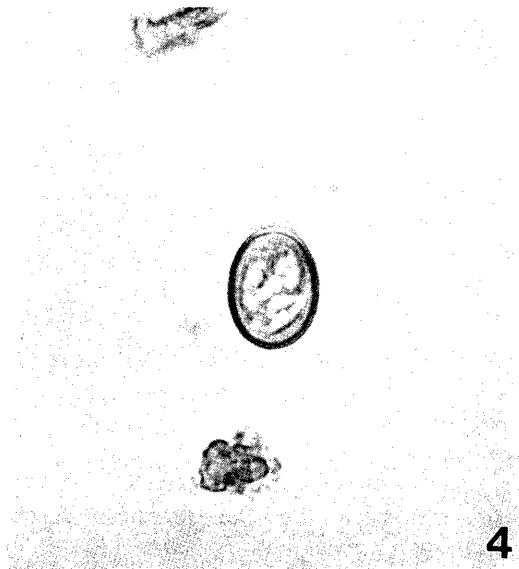


Fig. 4. *Eimeria naganoensis*. Sporulated oocyst with smooth wall and indistinct micropyle. $\times 200$.

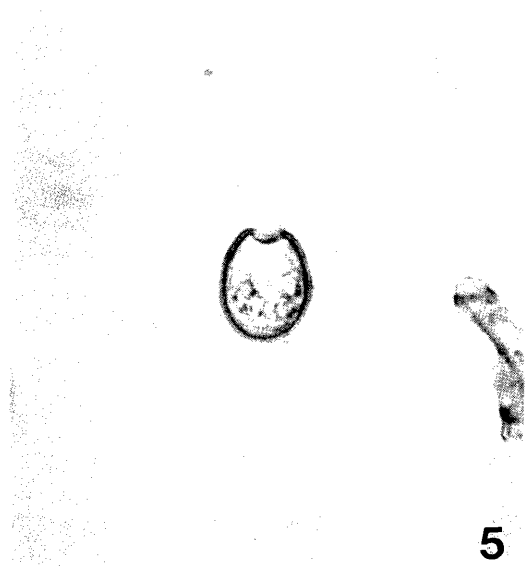


Fig. 5. *Eimeria naganoensis*. Incompletely sporulated oocyst with a distinct micropyle. $\times 200$.

without a micropyle and Stieda body. This species is also similar to *E. naganoensis*, but differs from it in the absence of micropyle and Stieda body. The oocyst of *E. yakimoff-matschoulskyi* Supperer and Kutzer, 1961 [9] is clearly distinguishable from the isolated four new species by the presence of a micropylar cap. The oocyst of *E. alpina* Supperer and Kutzer, 1961 [9] is distinctly distinguishable from other species by its characteristic spherical shape and small size. The oocyst of *E. suppereri* Kutzer, 1964 [2] is ellipsoidal and 45 by 34 μm in size. The oocyst wall is rough and composed of two layers; the outer layer is brown and the inner colorless or yellowish. The outer layer is relatively difficult to be separated from the inner layer. Sporulation time is 12 to 14 days. This oocyst is similar to that of *E. capricornis*, but differs in that the two layers of oocyst wall are difficult to be separated from each other and that the sporulation time is longer than that of *E. capricornis*.

In 1954, Ryšavý [6] reported other four species of *E. arloingi*, *E. crandallis*, *E. ninakohlyakimovae* and *E. parva* from the

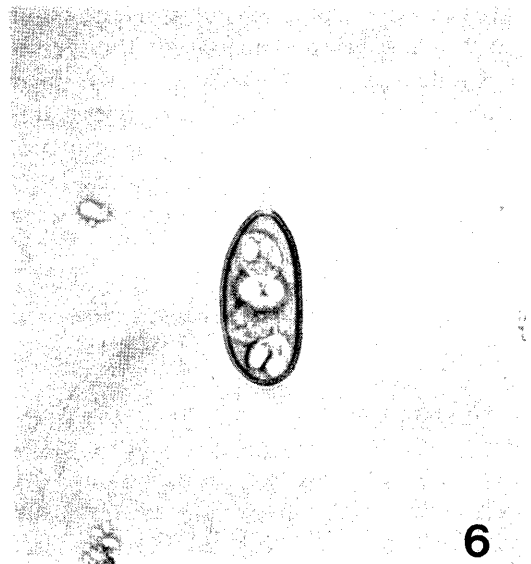


Fig. 6. *Eimeria kamoshika*. Sporulated oocyst with smooth wall and asymmetrical in shape. Micropyle is distinct. $\times 200$.

chamois. The natural hosts of these four species are domestic goats or domestic sheep. According to Levine and Ivens [3], any of the four species has not been reported from the chamois since 1954. As no

attempts have been made to carry out a cross-transmission experiment of these species since Ryšavý [6], it is not clear that the species are identical with those from their natural hosts. *Eimeria capricornis*, *E. nihonis*, *E. naganoensis* and *E. kamoshika* isolated by the author did not infect domestic goats. *Eimeria longispora* was isolated from the chamois by Rudovsky in 1922 [7], but after that Pellérdy [5] mentioned that no original description had been published on this species. So, it is considered that the name *E. longispora* will be nomina nudum.

The oocyst of *E. oreamni* Shah and Levine, 1964 [8] from the Rocky Mountain goat is similar to that of *E. nihonis*, however, *E. oreamni* can be distinguished from the *E. nihonis* by the features that a polar granule and thin Stieda body are present and that the outer layer of oocyst wall is smooth and yellowish. *Eimeria montanaensis* Todd and O'Gara, 1968 [11] is similar in oocyst size to *E. naganoensis*, but *E. montanaensis* has a polar granule and a micropylar cap. In addition, the outer layer of oocyst wall of this species is light blue. The oocyst of *E. ernsti* Todd and O'Gara, 1968 [11] can be morphologically distinguished from the present four new species by the presence of micropylar cap.

As mentioned above, the four newly isolated *Eimeria* species differed from those which have ever been reported from the saiga, chamois and Rocky Mountain goat. Coccidia should be identified not only by the morphology of oocysts but also by the development in the host. The Japanese serow, however, is designated a "special natural monument" by law. Therefore no examination is practicable with it. Hence, the author decided that the four species obtained from the Japanese serow are new

species from their morphological features of oocysts.

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要 約

ニホンカモシカから分離したコクシジウムの4新種：井上 勇（日本大学農獣医学部・医動物）——1982年12月から1984年3月までに本学解剖学研究室に搬入されたニホンカモシカ214頭について糞便検査を実施した。その結果4種類のオーシストを分離し、これらを既知オーシストと比較同定したところ新種であったので *E. capricornis*, *E. nihonis*, *E. naganoensis*, および *E. kamoshika* と命名した。*E. capricornis* は大きさ $48.02 \pm 0.53 \times 33.93 \pm 0.35 \mu\text{m}$, 濃褐色で卵円形, 外層は粗糙で濃褐色, 中層淡黄色, 内層は紫色であった。Micropylar cap. 外部残体, Stieda body は欠けていたが, micropyle, polar granule および内部残体は有しており胞子形成期間は6日であった。*E. nihonis* は大きさ $29.73 \pm 0.45 \times 21.01 \pm 0.29 \mu\text{m}$, 淡褐色で楕円形, 外層は粗糙であり褐色, 内層は黒色であった。Micropyle および内部残体は認められたがその他は欠けていた。なお, 胞子形成期間は3日であった。*E. naganoensis* は大きさ $20.04 \pm 0.05 \times 15.77 \pm 0.04 \mu\text{m}$, 淡褐色で卵円形, 壁は平滑で外層は淡黄色, 内層は紫色であった。Micropyle, 内部残体および小さな Stieda body を有し, 胞子形成期間は3日であった。*E. kamoshika* は大きさ $30.07 \pm 4.74 \times 14.58 \pm 2.03 \mu\text{m}$, 無色で長楕円形, 左右が不对称であった。壁は平滑で外層は淡黄色, 内層は紫色で, micropyle, 内部残体および小さな Stieda body を有し, 胞子形成期間は6日であった。