A NEW COCCIDIUM OF JAPANESE QUAILS: *EIMERIA UZURA* SP. NOV.

Kiyoshi TSUNODA

National Institute of Animal Health

Yaichi MURAKI

Japan Shaver Grandparent Poultry Farm, Incorporated

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Only a few reports have so far been published on the coccidia in Japanese quails (*Coturnix japonica*)^{8,11}. Even these reports deal only with observations on the oocysts of these parasites and the outbreak of coccidiosis due to spontaneous infection. Thus, no studies have been made on the life-cycle of these coccidia in any detail.

On the other hand, several morphological studies have already been made on the coccidia in quails. The quails investigated and discussed in published reports^{1,5,12}, however, were birds belonging to different genera from those of Japanese quails. It is, therefore, unjustifiable to apply the results of those previous studies directly to the coccidia in Japanese quails to be discussed here.

The Japanese quail is *Coturnix japonica*. The variety taxonomically close to this is *C. coturnix* which is commonly called a grey quail. Papers were published to describe two species of coccidia isolated from this quail in India^{2,3} and named *Eimeria coturnicis* and *E. bateri*. It deals, however, with the morphological aspect of a limited number of oocysts collected from the feces of quails spontaneously infected with these coccidia. Therefore, the data provided by these papers cannot be utilized for accurate comparison with coccidia in the other quails, especially the Japanese quail. The authors have isolated oocysts of coccidia with several different morphological patterns from those in the Japanese quail, and clarified the whole life-cycle of one of those coccidia. This coccidium was found to be a new species.

MATERIALS AND METHODS

Oocysts were collected from naturally infected Japanese quails. They were sporulated at 25°C in 2% aqueous solution of potassium bichromate. A single oocyst was then collected from among them by means of the agar plate method. It was then inoculated into a coccidium-free chick of Japanese quail 5 days of age. The newly generated oocysts thus obtained were incubated by the above mentioned method. After the sporulation was completed, approximately 2000 oocysts were again inoculated into six coccidium-free chicks of Japanese quail 12 days of age. From these chicks a large number of oocysts were collected for use in various tests.

Chicks of Japanese quail hatched at laboratory for use in the tests were kept in a chick breeder equipped with an electric heater. This breeder had been sterilized before use, so that chicks could completely be prevented from spontaneous infection with coccidia.

In order to examine the process of proliferation of the coccidium in the bodies of

Japanese quails, 2 to 4 Japanese quails were slaughtered each time for autopsy at certain intervals after the infection. Tissue samples were collected from them. Some of them were examined in a fresh, unstained state. Others were fixed and stained mainly with Giemsa's stain, Hematoxylin and eosin (HE), Heidenhain's and PAS stain. The intestinal tissues were fixed in neutral formalin, Gender's or Schaudinn's fixative and sometimes Bouin's fluid depending on the purpose of examination.

In order to determine the state of excretion of oocysts, especially their prepatent and patent periods, the following measures were used. On and after the third day after infection, fecal samples were collected twice a day and examined for the presence of oocysts by the sucrose floatation method. The time interval between infection and the first detection of oocysts was regarded as the prepatent period, while the patent period was defined as the time interval between the start of excretion of oocysts and the disappearance of oocysts from the feces in Japanese quails. The number of oocysts excreted into the feces was examined once a day and expressed in terms of O.P.G. In this test, the individual Japanese quail was transferred approximately every 20 hours into another cage sterilized before use, so that the bird might be prevented from possible re-infection with excreted oocysts in the same cage.

In the observation of oocysts, the sporulation time was defined as a term required for approximately 70% of the sporulation to be finished completely in 2% potassium bichromate solution at 25°C. The shape and size of the oocyst were measured 200 oocysts which had been collected from a Japanese quail infected with a single oocyst.

In order to determine the range of hosts for this coccidium, the cross infection test was conducted using chicks of several species of birds relatively close to the Japanese quail from a taxonomical point of view. These species of birds were the copper pheasant (*Syrnaticus soemmerringii*), The Japanese pheasant (*Phasianus versicolor*), the Chinese bamboo partridge (*Bambusicola thoracica*), The Guinea fowl (*Numida galeata*), the turkey (*Meleagris gallopago*), the chicken (*Gallus gallus*), the bobwhite (*Colinus virginianus*), the golden pheasant (*Chrysolophus pictus*), the painted quail (*Excalfactoria chinensis*), the California valley quail (*Lophortyx californica*), and the ptarmigan (*Lagopus mutus*).

RESULTS

Oocysts:

The shape of oocysts of *E. uzura* was broad ellipsoid or semi-oval. The wall was almost colorless. The sporont was slightly yellowish brown in color. The oocysts were 18.75 to 30.0 μ in length and 15.0 to 23.75 μ in width. Their average size was $24.39 \times 18.66 \mu$ (Fig. 1). The polar inclusion was for the most part single (Table 1). Each oocyst had no oocystic residual body, but a sporocystic residual body which consisted of granules forming a small mass was found in each sporocyst. There was no micropyle in either unsporulated or sporulated oocyst. However, when the small intestine was examined for the presence of oocysts 3 to 6 hours after inoculation, the micropyle was found clearly appearing at one end of the oocyst (Fig. 2).

The sporulation time was 22 to 24 hours at 25°C. During the sporulation the pyramid stage was found to be equilateral triangle in shape.

Localization:

The main parasitic location of this *Eimeria* was in the duodenum. In the case of heavy infection, a minor extent of parasitism of this *Eimeria* was found in the upper part of the small intestine, but very seldom in the lower part of the small intestine. No

Fig. 1. Frequency distribution of size of oocysts

Fig. 2. Micropyle of oocyst

parasitism was observed in the ceca or rectum.

Prepatent and patent periods:

The prepatent period of this species, *E. uzura*, was found to be 88 to 96 hours, or around 4 days. The length of this period was fixed in Japanese quails, irrespective of age (in days), sex, and the variety of infection. The patent period of this species was found to be from 4 to 16 days, or 13 days on the average, after infection. There were differences ranging from 1 to 2 days in the period required for oocysts to disappear from the feces among individual Japanese quails. There was, however, hardly any difference in the patent period between the severe infection and the mild one. On the other hand, two peaks were observed in the O.P.G. value during the period, one from the 5th to the 6th days and the other from the 10th to the 13th days after infection. They seem to show that there may have been two occasions for intensive gametogony (Fig. 3).

Endogenous life-cycle:

The life-cycle of *E. uzura* in the small intestine of the Japanese quail was as follows. When inoculated into a Japanese quail orally, oocysts underwent excystation releasing sporozoites, in the duodenum and the upper part of the small intestine in 1.5 to 5.0 hours. On this occasion, it was through a micropyle formed at one end of the oocyst that sporozoites were usually released.

The size of the sporozoites thus released was $9-12.0 \times 3.0-4.8 \mu$, or $10.03 \times 4.46 \mu$ on the average, in specimens stained with Giemsa's stain. A nucleus was present near the obtuse end of the body. The nucleus was usually stained in a reddish violet color. At the center of the body, there was a vacuole extending over approximately $1/2$ the length of the body. These were strongly characteristic differences between the sporozoites and the merozoites. This vacuole stained light yellow with Giemsa's stain. It showed evidently eosinophilic affinity when stained with HE. The released sporozoites immediately penetrated the epithelial cells of villi in the small intestine and turned to be spherical in shape. No further progress took place beyond this state for approximately 24 hours after infection. When 48 hours had passed after the infection, almost all the sporozoites were found to have grown up into young schizonts and some sporozoites

Table 1. The number of polar inclusions of oocysts of *Eimeria uzura*.

No. of polar inclusions	No. of oocysts	%
0	49	12.25
1	232	58.00
2	87	21.75
3	29	7.25
4	3	0.75

} 87.75%

Fig. 3. Patent period of *E. uzura*. Comparison between heavy and slight infection

Heavy infection — 9.2×10^6 oocysts

Slight infection - - - 3.4×10^2 oocysts

further into those of mature state containing 6 to 12 merozoites. The young schizonts usually had 2–6 nuclei and were $5.4\text{--}6.5 \times 5.9\text{--}6.5 \mu$ in size, or $6.13 \times 6.14 \mu$ on the average, when stained with Giemsa's stain. Their cytoplasm could be stained into a dark blue color and their nuclei into a reddish violet color. This tinctorial property was the same as the stainability of many of the other coccidia.

Numerous mature schizonts, young schizonts, and the released merozoites were observed in the duodenum and the upper part of the small intestine 72 hours after infection. From this result, it was made clear that schizogony took place rapidly within 48 to 72 hours after infection.

The cytoplasm of mature schizonts could be stained into a dark blue color with Giemsa's stain, while the nuclei of individual merozoites could be stained into a reddish violet color. Merozoites were formed in schizonts by the preceding karyostenosis. Then the nuclei aggregated around the cytoplasm and protruded from the cytoplasm of the schizonts to form merozoites. Therefore, these protozoa multiply by asexual reproduction, provided with the same kind of germination system as the other coccidia.

Mature schizonts were $10.5\text{--}12.7 \times 10.0\text{--}11.8 \mu$ or $11.87 \times 10.7 \mu$ on the average when stained with Giemsa's stain. Merozoites released from them were divided into 2 types, a large and a small, 72 hours or more, particularly 96 hours, after infection. The larger merozoites were $8.6\text{--}9.6 \times 2.9\text{--}3.4 \mu$ or $9.1 \times 3.2 \mu$ on the average and the smaller ones $5.0\text{--}5.9 \times 1.0\text{--}2.0 \mu$ or $5.3 \times 1.4 \mu$ on the average.

The stainability of the larger merozoites was such that the cytoplasm of these merozoites was stained darker with Giemsa's stain than that of the smaller ones. When 78 to 80 hours had passed after the inoculation, gametocytes began to be formed. Zygotes were detected already 88 hours after the inoculation, and a small number of them were found to have grown into oocysts.

In specimens stained with Giemsa's stain 120 hours after infection, mature macrogametocytes were $9.8-12.5 \times 9.2-12.0 \mu$, or $10.6 \times 10.6 \mu$ on the average, in size. Most of them were spherical in shape, with their cytoplasm stained in a dark blue color. Around the center of the cytoplasm a nucleus could be detected.

The mature microgametocytes were $14.8-19.5 \times 15.0-16.0 \mu$, or $17.15 \times 15.56 \mu$ on the average, in size. Their cytoplasm was stained in a light blue color. Thinctorially, the cytoplasm was characterized by a lighter color than that of macrogametocytes or schizonts.

Although microgametocytes, particularly young ones, resembled schizonts in shape, the nuclei in the cytoplasm of the former could be stained more clearly than those of merozoites in the schizonts. Hence, distinction between the microgametocytes and the young schizonts was not so difficult. A mature microgametocyte had a residual body at the center. There were a number of crescent microgametes between it and the cyst wall. Each microgamete had 2 long flagella and was weakly stained into a reddish violet color. The flagella could be seen more clearly under the phase contrast microscope.

The zygotes were $14.0-17.2 \times 14.0-16.2 \mu$, or $15.28 \times 15.08 \mu$ on the average, in size. Their stainability was generally weakend in Giemsa's stain. A number of granules uniform in size appeared in the cytoplasm. They could be stained with eosin. Therefore, the granules presented a light orange color when stained with Giemsa's stain. In

Table 2. Results of cross infection test of *E. uzura*.

Experimental bird	Infection with <i>E. uzura</i>	Experimental Eimeria	Infectivity to Japanese quail
<i>Meleagris gallopago</i>	—	<i>E. meleagridis</i>	—
		<i>E. meleagritidis</i>	—
		<i>E. adenoides</i>	—
<i>Phasianus versicolor</i>	—	<i>E. dispersa</i>	—
		<i>E. phasiani</i>	—
<i>Numida galeata</i>	—	<i>E. numidae</i>	—
<i>Gallus gallus</i>	—	<i>E. tenella</i>	—
		<i>E. necatrix</i>	—
		<i>E. acervulena</i>	—
		<i>E. maxima</i>	—
		<i>E. brunetti</i>	—
		<i>E. praecox</i>	—
		<i>E. mitis</i>	—
<i>Chrysolophus pictus</i>	—	<i>E. sp</i> (<i>E. dispersa</i> ?)	—
<i>Syrnaticus soemmerringii</i>	—	<i>E. sp</i> (<i>E. dispersa</i> ?)	—
<i>Bambuscicola thoracica</i>	—	<i>E. sp</i>	—
<i>Colinus virginianus</i>	—	<i>E. sp</i> (<i>E. dispersa</i> ?)	—
<i>Excalfactoria chinensis</i>	—	<i>E. sp</i>	—
<i>Lophortyx californica</i>	—	<i>E. sp</i> (<i>E. dispersa</i> ?)	—
<i>Lagopus mutus</i>	*	<i>E. sp</i>	—
<i>Coturnix japonica</i>	+	<i>E. uzura</i>	+

—: Not infected

+: Infected

*: Not tested

addition to them, there were many granules uneven in size which could be stained into a blue to reddish violet color. All these asexual and sexual bodies could be observed only in the epithelial cells of the villi of the duodenum and the upper part of the small intestine.

The sporozoite was always negative for the PAS-staining reaction. A small number of merozoites had positive granules at the blunt end of the cytoplasm, although the greater number of them had not those granules. Schizonts, especially young one, were negative for staining. In mature schizonts, sometimes a small number of them stained to be weakly positive. Microgametocytes, young and mature, were negative for staining. Young macrogametocytes were negative, as well as the microgametocytes. However, matured ones and the zygotes showed a positive reaction. Moreover, they became more intense in stainability with the advance in development. In the oocyst, the wall and the sporont presented strong stainability. The reaction of *E. uzura* to the PAS staining was comparable to that of *E. acervulina* in chickens.

Cross infection test:

A series of tests were conducted to infect chicks of Japanese quails with various oocysts obtained from such birds as relatively close to the quails and, in reverse to the above, to infect chicks of these birds with oocyst of *E. uzura*. If *E. uzura* was not specific to Japanese quails, infection would take place in one or more of these birds, or an *Eimeria* species derived from some of those birds would cause parasitism in Japanese quails. Table 2 shows the results thus obtained and indicating that *E. uzura* is specific to the Japanese quail.

Pathogenicity:

E. uzura was not so pathogenic to Japanese quails. Its pathogenicity was somewhat similar to that of *E. acervulina* to chickens. When young quail chicks 3 to 5 days of age were infected with more than 100,000 oocysts, they manifested diarrhea, anemia, and emaciation 5 to 8 days after infection. When quail chicks more than 2 weeks old and adult quails were infected in the same manner, only moderate clinical symptoms could be seen in them.

DISCUSSION

Studies have not so far been made in details on the coccidia of Japanese quails. Only a few reports have been made on spontaneously infected Japanese quails. Hence, nothing has been established to date on the species of coccidia infecting Japanese quails or on the properties of these coccidia. In the present examination, the authors succeeded in isolating some oocysts differenting size and shape. Furthermore, they isolated one particular type from these oocysts by the single oocyst inoculation method. Through this experiment the life-cycle of the coccidia infecting Japanese quails could be made clear. In India, CHAKRAVARTY³⁾ isolated a species of *Eimeria* from grey quails, which are taxonomically close to Japanese quails, and named the species *Eimeria coturnicis*. After that, BHATIA et al.²⁾ also isolated another type of *Eimeria* from the grey quail and named it *E. bateri*. The oocysts of these 2 species resembled that of *E. uzura* in shape. However, the oocysts of *E. coturnicis* measured $26.4\text{--}38.8 \times 19.8\text{--}26.4 \mu$ in size, those of *E. bateri* $17.0\text{--}31.0 \times 13.0\text{--}22.0 \mu$ and those of *E. uzura* $18.75\text{--}30.00 \times 15.00\text{--}23.75 \mu$. From these data it was obvious that the oocysts of *E. coturnicis* were larger than those of *E. uzura* and *E. bateri*, but those of the latter 2 species were more similar in size or shape. The oocysts of *E. uzura* usually had one or more oocystic inclusions, but *E. coturnicis* had no such inclusions. Moreover, the oocysts of *E. coturnicis* and *E. bateri* had no micropyle, but those of *E. uzura* had one.

The presence or absence of micropyle could be considered one of the important points for the taxonomy of *Eimeria*.

It was confirmed that several different species of *Eimeria* had infected Japanese quails. Based on this finding, it was assumed that several species of *Eimeria* might also cause parasitism in grey quails. Unfortunately, at present in Japan, it is difficult to obtain grey quails for examination. It seems to be justified to classify the *Eimeria* which was isolated in the present studies into a new species in view of the results of observation on its whole life-cycle and the characteristics of oocysts. Therefore, it is considered that the name *Eimeria uzura* Tsunoda 1790 should be given to this *Eimeria*, since "uzura" stands for the quail in Japanese.

On the other hand, it has been mentioned in some reports that of the species of *Eimeria* which attack the gallinaceous birds, only *E. dispersa*^{1,4-6,9-11,12)} is infective to 8 different species of birds, including *C. coturnix*. Accordingly, it is clear from these findings that *E. dispersa* is very peculiar among the species of *Eimeria*, provided that no previous investigators have failed to make sure to protect their experimental birds perfectly from natural infection with coccidia.

In the present experiments, *E. dispersa* which had been isolated from Japanese pheasants was never infective to Japanese quails. On the contrary, *E. uzura* was never infective to Japanese pheasants. Therefore, *E. uzura* was proved to be a species different from *E. dispersa*.

SUMMARY

A species of coccidium belonging to the genus *Eimeria* was isolated from Japanese quails. A pure strain of this species was obtained by the single oocyst infection method. Then the whole life-cycle of this species was clarified by experimental infection. This species was affirmed to be a new one and named *Eimeria uzura*.

E. uzura was mainly located in the duodenum and the upper part of the small intestine. This species resembled *E. coturnicis* and *E. batri* in the morphology of the oocyst except the presence of the micropyle.

The sporulation time of *E. uzura* was 22–24 hrs. at 25°C. The prepatent period was 88–96 hrs. after infection and the patent period was around 13 days.

Detailed examination, however, revealed some important differences among these three species. Experimental infection with *E. uzura* failed in 11 different species of birds which are taxonomically close to the Japanese quail. Twenty species of *Eimeria* which attacked the 11 species of birds were never infective to the Japanese quail.

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日本ウズラから分離したコクシジウムの1新種 *Eimeria uzura*

角 田 清

農林省家畜衛生試験場

村 木 八 一

日本シェーバー原種鶏農場

(昭和45年12月15日受付)

日本ウズラ (*Coturnix japonica*) のコクシジウムについては、報告がとぼしく、自然発生例の観察を一二みるのみである。いっぽう quails といわれている鳥のコクシジウムについては、いくつかの報告がある。しかし、これは日本ウズラとは全く属を異にする鳥であるから、それらのコクシジウムと日本ウズラのものとは、全く異なるものであることは、*Eimeria* 属の宿主特異性からみても、容易に考えられることである。

筆者らが日本ウズラから純分離に成功した1種の *Eimeria* は、インドで灰色ウズラ (*C. coturnix*) のコクシジウムとして報告された *Eimeria coturnicis*, および *E. bateri*, とくらべて、オーシストの形は似ているが、その大きさ, polar inclusion, および micropyle の有無などからみても、明らかに異なる種類であるとみられる。*E. coturnicis* は、特に自然感染例の糞中の少数のオーシストの形態をみているに過ぎないため、はたして、これが1種類のコクシジウムのオーシストを観察したものか否かは疑がわしい。

今回分離したコクシジウムについては、単一オーシスト感染法で純分離した株を用いて、その全発育環を明らかにした。成熟オーシスト感染後1.5~5時間でスポロゾイトは遊出し、十二指腸および小腸上部の絨毛上皮細胞内に侵入して、球形

となる。その後、初代シゾントは22~48時間で、2代以降のシゾントは48時間以降に、形成される。ガメトサイトは88~96時間の間に形成され、新生のオーシストが糞中に初めて排泄されるのは、感染後4日目である。シゾントおよびガメトサイトは、すべて十二指腸および小腸上部の絨毛上皮細胞内で増殖、発育し、決して固有層以下には侵入しない。また、宿主細胞の核の上部の細胞質内でのみ増殖する。これは鶏の *E. acervulina* と良く余似た性質を有する。オーシストは、25°Cで Sporulation time が24時間である。また、patent period は、濃、軽感染を問はず、13日間であった。

また、ウズラに近縁な鳥類11属について、*E. uzura* のオーシストで感染試験を行なったが、いずれも感染は成立しなかった。さらにこれらの鳥類から分離した少なくとも20種のコクシジウムのオーシストを日本ウズラに感染させたが、いずれも感染しなかった。とくに、*E. dispersa* は、各種の鳥類に感染する可能性があることが報じられているが、日本ウズラには感染しなかった。

以上の実験結果からみて、今回分離したコクシジウムは、*Eimeria uzura* TSUNODA 1970, なる種名を与え、新種として扱うのが適当と考えられる。

EXPLANATION OF PLATES

PLATE I

- 1) Unsporulated oocyst. $\times 1,200$.
- 2) Sporulated oocyst. $\times 1,200$.
- 3) Liberated sporozoite 3 hours after infection. $\times 1,200$.
- 4) First-generation schizont 48 hours after infection. $\times 1,200$.
- 5) Second-generation schizont (not completely mature) 72 hours after infection. $\times 1,200$.
- 6) Merozoites of small type 72 hours after infection. $\times 1,200$.
- 7) Macrogametocyte 96 hours after infection. $\times 1,200$.
- 8) Microgametocyte 120 hours after infection. $\times 1,200$.
- 9) Zygote 120 hours after infection.
- 3) to 9): Preparations stained with Giemsa's stain.

PLATE II

- 10) Sporozoites in epithelial cells 5 hours after infection. $\times 600$. (HE).
- 11) Immature first-generation schizont in the epithelial cell 24 hours after infection. (HE), $\times 600$.
- 12) Mature first-generation schizonts 48 hours after infection. (HE), $\times 600$.
- 13) Second-generation schizonts located above the nuclei of epithelial cells 72 hours after infection. (HE), $\times 600$.
- 14) Micro- and Macrogametocytes in epithelial cells 96 hours after infection. PAS and hematoxylin, $\times 600$.
- 15) Mature oocysts and zygotes 120 hours after infection. PAS and hematoxylin, $\times 600$.