日本産Eimeria tenella株間のプリパテント・ピリオドの変異

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Variability of Prepatent Period in Japanese *Eimeria tenella* Strains

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The prepatent period has been considered to be relatively constant for a definite species of coccidia and can be used as one of the criteria for identification [2, 5, 7]. However, various lengths of the prepatent period of *Eimeria tenella* have been reported; 96 hr [5], about 5 days [9], 138 hr [3], 6 days [8], and 7 days [2, 14]. Recently, some precocious coccidian strains were developed in laboratories [4, 6, 11–13] which indicated that the prepatent period could be experimentally changed.

In this study, we observed prepatent periods of 3 field-isolated and 2 laboratory-maintained strains of *E. tenella* and also examined the period of a field-isolated strain after serial passages.

Strain A, derived from the National Institute of Animal Health, Japan, had been passaged in our laboratory for about 8 years by inoculating chickens with oocysts from the feces discharged 7, 8 and 9 days after inoculation. Strains B, isolated in Kanagawa prefecture of Japan 8 years ago, had been maintained in our laboratory. Strains C, D and E were recently collected from Miyazaki, Okinawa and Ibaraki prefecture of Japan, respectively and cloned cultures were established by the agar plate method [15]. Laboratory-maintained strains were prepared by the single oocyst method prior to the experiment. Day-old white Leghorn-cross (Dekalb: commercial name) cockerels were purchased from a commercial hatchery and kept in wire-floored metal cages in an isolated poultry house until use. After the inoculation with oocysts, chickens were individually reared. During the course of experiments, chickens were fed water and basal ration *ad libitum*. On day 12 after birth, each chicken received 10^3 oocysts in the crop with a pipette. Sporulated oocysts were prepared within 2 months prior to inoculation. From 96 hr after the inoculation, feces of each chicken were collected hourly to examine oocysts by the flotation method with saturated NaCl solution. Trails with each coccidium strain were independently carried out twice using 3 birds each. During the experimental period feces of 2 non-infected control birds were also collected daily for the examination of oocysts. These control and infected birds were necropsied at the end of experiments to examine coccidian parasites in their mucosa of digestive tracts. The data were statistically analyzed by Student' *t*-test.

In the present experiment, the shortest prepatent period in the five *E. tenella* strain was 122.0 hr of the Strain B, one of the laboratory-maintained strains. The longest one was 142.8 hr of Strain E, one of newly isolated field strains. The difference between these two values was highly statistically significant ($p<0.001$). Although two trials for each strain were performed independently, intra-strain difference in the prepatent period was not so large as inter-strain difference among the strains. The largest difference between two trails was 7 hr (Strain C) (Table 1). Neither protozoa in the mucosa of digestive tract nor oocyst in the feces was detected in control birds. From the present results which indicated variance in the prepatent period among the strains of *E. tenella*, it was concluded that other characters such as endogenous development should be also taken into consideration for identification of *E. tenella* in the field.

Strain C was divided into 2 lines, the day-6 line and day-13 line, by harvesting oocysts on two different days in the patent period. Oocysts of the day-6 line were obtained from feces of 5 birds from 144 hr to 152 hr (6th day) after the infection, and those of the day-13 line were obtained from 327 hr to 335 hr (13th day). Oocysts were collected by the flotation method with saturated NaCl solution, sporulated in 2.5% potassium dichromate solution at 25°C, and then preserved at 4°C until use. In each passage, OPD values (oocysts production per day) were calculated daily by *Stool's* method.

At the sixth passage, the prepatent period of the day-13 line was 132.3 hr, which was prolonged significantly compared with both those of the parent strain (124.7 hr) and those of of the day-6 line (127.3 hr) ($p<0.01$). The oocysts

Table 1. Prepatent periods of *Eimeria tenella* strains

Strain	Prepatent period(hr)	
	Range	Mean±SE
A	132–135	133.7±0.50
B	120–124	122.0±0.58
C	120–127	124.7±1.02
D	133–135	133.8±0.33
E	140–144	142.8±0.60

Each group consisted of six birds.
SE: Standard error.

Table 2. Prepatent periods of lines at 6th passage (day-6 and day-13 lines) and the parent line (Strain C)

Line	Prepatent period(hr)	
	Range	Mean±SE
Day-6	127–128	127.3±0.521
Day-13	130–134	132.3±0.61
Parent	120–127	124.7±1.02

Each group consisted of six birds.
SE: Standard error.

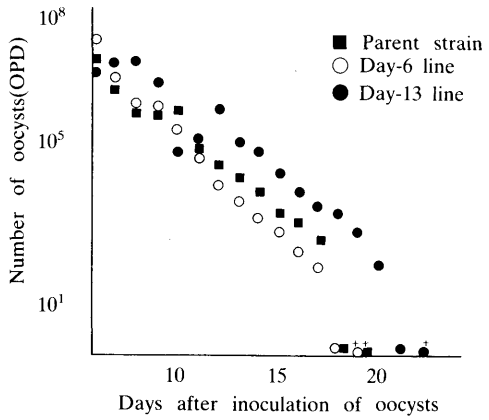

Fig. 1. Oocyst-output of chickens inoculated with oocysts passaged 5 times (Day-6 and Day-13 lines) or once (Strain C; Parent).
+: Oocysts were detected by the flotation methods. Each line consisted of 5 birds.

output period was also prolonged in the day-13 line. The oocyst output pattern of the day-6 and the day-13 lines tended to differ from that of the parent strain (Table 2 and Fig. 1).

The prepatent period of the day-13 line might be prolonged easily because the selection pressure against the day-13 line is stronger than that against the day-6 line.

As the lowered pathogenicity of precocious lines has been reported by several authors [1, 4–6], the pathogenicity of the prepatent period prolonged line should be investigated.

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要　　約

日本産 *Eimeria tenella* 株間のプリパテント・ピリオドの変異（短報）：斎藤康秀・板垣　博（麻布大学獣医学部寄生虫学教室）——*E. tenella* の野外株3株および研究室株2株のプレパテント・ピリオドには平均122.0～142.8時間の株間の差がみられ最短期間と最長期間の間には統計的に有意差がみられた．野外株の1株を，パテント・ピリオドの前半期および後半期にオーシスト回収を繰り返すことにより2系（6日系および13日系）に分け，それぞれを継代したところ13日系では6継代目でプリパテント・ピリオドに7.6時間の有意な延長がみられたが，6日系では変化がみられなかった．