Further Investigation of the Pathogenicity of a Chicken Embryo Passage Line of *Eimeria tenella*

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Attenuated lines of *Eimeria mivati* [5, 7], *E. tenella* [2, 4], and *E. necatrix* [2, 16] were attained by serial passage of the parasites in chicken embryos. We have serially passaged a strain of *E. tenella* of Japan origin in chicken embryos 51 times. No changes in reproductivity and lethality of the passage line were observed in chicken embryos, but when this line was inoculated to chickens, less severe cecal lesions and hemorrhage with better body weight gain, feed intake and feed requirement were observed as compared to the infection of the original strain [13].

Periodic acid-Schiff (PAS)-positive granules were found in sporozoites of *E. tenella* [3, 18], and characterized as amylopectin [10, 15]. In the original strain of *E. tenella* which was used for this passage work, amylopectin contents of the sporozoites were found to be related to abilities to survive, actively move, and invade and develop in host cells [9, 14]. Amylopectin of *E. tenella* sporozoite has been considered to have an important role for the successful infection [14, 17]. In the present study for the purpose of finding out causes of the changed pathogenicity of the passage line, amylopectin contents of the

sporozoites were investigated firstly. Secondly, the endogenous development of the line was compared with that of the original strain in chickens.

E. tenella K-2 strain and the chicken embryo passage line were used [8, 11]. Oocysts from a culture serially passaged 50 times (P50) and once (P1) were prepared under the same condition in chicken embryos after inoculation with sporozoites from oocysts of a culture serially passaged 49 times in chicken embryos and of the original strain from chickens, respectively. Oocysts of P50 and P1 were prepared each time in three different experiments. Sporozoites were stained with PAS stain, and each sporozoite was observed microscopically and classified into six PAS indices (from 0 to 5) on the basis of amylopectin granule contents. The mean value of PAS indices of 100 individual sporozoites in each sample was defined as PAS index [9].

PAS indices of P50 sporozoites freshly prepared by *in vitro* excystation were 80-90% of those of P1 (Table 1), indicating significantly smaller amount of amylopectin granules.

Sporozoites prepared in the experiment 3 in Table 1 were suspended in phosphate buffer solution and incubated at 37°C, and their amylopectin contents were examined (Fig. 1). PAS

Table 1. Amylopectin contents of fresh sporozoites of *E. tenella*

Experiment number	P50 ^{a)}	P1	P50/P1 ^{b)} (%)
1	3.60±0.07	4.03±0.01 ^{*c)}	89
2	3.28±0.08	4.12±0.03 ^{**}	80
3	4.38±0.12	4.87±0.05 [*]	90

a) PAS index of sporozoites of cultures passaged 50 times (P50) or once (P1) in chicken embryos; Mean±SE (n=3).

b) PAS index of P50/PAS index of P1.

c) There were significant differences between indices of P50 and those of P1 (*: P<0.05, **: P<0.01).

Fig. 1. Amylopectin contents of sporozoites of *E. tenella*. Sporozoites of cultures passaged 50 times in chicken embryos (P50) or once (P1) were incubated in phosphate buffer solution at 37°C, and stained with PAS stain. PAS index was calculated on the basis of amylopectin granule contents. The number of sporozoites of P50 was not enough for the calculation of the index at 8 hr incubation.

indices of P50 and P1 decreased in proportion to incubation period. At 6 hr incubation, sporozoites of P50 contained a small amount of amylopectin (PAS index: 0.9), and 50% of them did not have amylopectin granules at all. At 8 hr incubation, sporozoites of P1 still contained several numbers of amylopectin granules, and showed higher value of PAS index (2.3) than that of P50 sporozoites at 4 hr incubation (2.2). The number of sporozoites of P50 was not enough for the calculation of the index at 8 hr. Many of them might autolyze. In the previous work [9], it was observed that sporozoites with scarce amount of amylopectin lost viability and disappeared by autolysis. These data indicate that sporozoites of the passage strain contain smaller amount of amylopectin at the time of excystation and they consume amylopectin and lose viability in a shorter period as compared with the original strain. By mutation or selection, synthesis or metabolism of amylopectin of the sporozoites might have changed in the course of passage in chicken embryos.

Farr *et al.* [1] mentioned that free sporozoites were detected at the middle part of the small intestine at 1 hr after inoculation of *E. tenella* oocysts and in cecum at 3 hr after inoculation. In our preliminary observation (Nakai and Ogimo-

to; unpublished data), sporozoites were detected in cecum at 2 hr after inoculation of oocysts. Relatively higher number of sporozoites, however, were observed in cecum at 8 hr after inoculation. These observations suggest that sporozoites reach cecum in 1 or 2 hr after excystation, and many of them remain in the lumen of cecum for several hours before invasion to host cells. Sporozoites were shown to metabolize exogenous sugars as the substitution of amylopectin reserve [11, 12]. When little amounts of sugars or some substrates are available in intestine, sporozoites have to survive by using own amylopectin reserve as the energy source. Sporozoites of the passage line may consume amylopectin reserve while moving down and staying in intestine and cecum, and many of them may lose viability or ability to infect host cells.

Endogenous development of the passage line was studied in chickens. Oocysts of a culture serially passaged 51 times in chicken embryos (P51) and once (P1) were prepared as mentioned. Fifteen day-old White Leghorn male chickens were inoculated with 1×10^4 oocysts of P51 or P1, and sacrificed on day 2, 4, 6 and 7 after inoculation. Development stages of the parasites in ceca were investigated after hematoxylin-eosin stain or PAS stain. First-generation schizonts were seen in epithelial cells of the crypts of Lieberkuehn on day 2 after inoculation of P51 or P1. In both cases of infection of P51 and of P1, large immature and mature second-generation schizonts were seen in the tunica propria on day 4. Tissue damage was also seen in both cases, associated with large second-generation schizonts. Numerous gametes were seen in epithelial cells of the crypts on day 6 after inoculation of P51 or P1, and numerous oocysts were seen on day 7. There were no differences between P51 and P1 in morphology of the first- and second-generation schizonts and merozoites, gametes and oocysts. Parasitized areas of each stages of P51 in cecal tissues were not different from those of P1 either. Characters of the passage strain did not correspond to those of *E. tenella* chick embryo-adapted lines of Long [6] and Gore *et al.* [2] which had characteristically small size of second-generation schizonts.

Sporozoites of the chicken embryo passage line induced the lower pathogenicity in chickens, regardless of the fact that the passage line has the

same endogenous development stages in cecal tissues as those of the original strain. It is considered to be the difference in amylopectin contents of the sporozoites. The sporozoites of the passage strain consume amylopectin reserve quickly while moving down intestine and cecum, and a small population of sporozoites with enough amylopectin reserve are able to infect. In chicken embryos, smaller amount of amylopectin reserve of the passage strain may not affect the infectivity of the sporozoites, because sporozoites can reach host cells before consumption of amylopectin reserve in the allantoic cavity. Therefore, low pathogenicity of the chicken embryo passage line to chickens might be related to the smaller amylopectin reserve in the sporozoites.

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要 約

鶏胚内継代株 *Eimeria tenella* の病原性(短報)：中井 裕・扇元敬司¹⁾(茨城大学農学部家畜衛生学教室，¹⁾東北大学農学部家畜衛生学教室)——鶏胚内で50代以上継代した *E. tenella* は、鶏胚を1回通過させたものと比較して、ヒナ体内での感染部位や増殖像に差はなかったが、脱殻直後の sporozoite の持つアミロペクチン顆粒が少なく、sporozoite を培養すると早期にこの顆粒が消失した。sporozoite がアミロペクチンを十分持たないことが鶏胚内継代株のヒナに対する病原性の減弱化の一因と推測された。