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The Comparison of *Isospora bigemina* Large Type of the Cat and *Besnoitia wallacei*

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ABSTRACT. *Isospora bigemina* large type of cats was compared with *Besnoitia wallacei*. *B. wallacei* was slightly infective to hamsters as well as *I. bigemina* large type. The former was not different to the latter in size of schizonts. Both protozoa were coincident serologically and immunologically. They were found to be identical.—**KEY WORDS:** *Besnoitia wallacei*, *Isospora bigemina*.

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Previously, we reported the isolation of *Isospora bigemina* large type oocysts from a cat, and the biological characteristics of this protozoa, such as structure, size, multiplication and distribution of each developmental stage, the life cycle, and so on [2]. As the results, it was clarified that this protozoa was very similar to *Besnoitia wallacei* [1, 3] in various characteristics. Namely, the intermediate hosts are mice and rats, the final host is cats, and the life cycle is an obligatory heteroxenous in both protozoa. The structure and size of oocysts and cysts, distribution of the endogenous stages, and the prepatent period of *I. bigemina* large type are closely similar to those of *B. wallacei*. Especially, *I. bigemina* large type also forms *Besnoitia*-like cysts in several organs of the intermediate hosts.

Both protozoa, however, were not always coincident in all characteristics. For example, we found that rabbits, voles and mongolian gerbils also played as the intermediate hosts of *I. bigemina* large type, but in *B. wallacei* these animals were not examined. The oocysts of this protozoa were slightly infective to hamsters, whereas those of *B. wallacei* were reported to be not. Moreover, the size of matured schizonts in the intestine

of cats was $50-120 \times 35-80 \mu\text{m}$ in *I. bigemina* large type, whereas that of *B. wallacei* was reported to be $500-800 \mu\text{m}$ in diameter. Therefore, we considered that it is necessary to compare both protozoa in detail.

Fortunately, we had a chance to receive *B. wallacei* oocysts from Dr. Frenkel by his kindness and to compare both protozoa. In the present paper, *B. wallacei* from Dr. Frenkel will be called the Hawaii strain, and *I. bigemina* large type called the NIAH strain.

Infectivity to hamsters of the Hawaii strain was examined. First, eight conventional hamsters (Nos. 1–8), which were free from coccidia, were inoculated orally with $1.5-3.0 \times 10^4$ oocysts of the Hawaii strain. When they were killed 34 days after inoculation, they had no cysts detected microscopically in their organs. Four cats fed the organs of these hamsters did not discharge any oocysts. Second, the other 9 hamsters (Nos. 9–17) were also inoculated orally with $2.0-4.0 \times 10^5$ oocysts of the Hawaii strain. When they were killed 62 and 63 days after inoculation, they were also cyst negative in microscopic examination with various organs of them. When four cats were given 4 of these 9 hamsters, however, two of 4 cats discharged oocysts 18 and 21 days after in-

gestion. These results are summarized in Table 1.

Thus, the hamster was found to be slightly susceptible to the Hawaii strain as well as the NIAH strain. Rabbits were also susceptible to the Hawaii strain and used for the serological examination later.

Matured schizonts of the Hawaii strain were compared with those of the NIAH strain.

In our experiments, matured schizonts of the Hawaii strain in the intestine of cats usually measured $45-125 \times 40-100 \mu\text{m}$, with the largest size of $150 \times 88 \mu\text{m}$. We could not find such large schizonts measured $500-800 \mu\text{m}$ in diameter as described by Frenkel [1]. The size of schizonts of the Hawaii strain was not different from that of the NIAH strain.

Moreover, both strains were compared serologically by the agar gel diffusion and direct fluorescent antibody (FA) tests. Rats inoculated with oocysts of the Hawaii strain or the NIAH strain, respectively, were killed for preparing antigens. The lungs were minced, added veronal buffered saline, mortared, made into tissue suspension, repeated 7 times freezing and thawing, and centrifuged at 10,000 rpm for one hour. Then the supernatant was used as an antigen of the agar gel diffusion test. Sera of mice, rats and rabbits infected with oocysts of the Hawaii strain or the NIAH strain were used for the test. Each antigen was put into center well, and six sera of a mouse, rat and rabbit infected with the

each strain were put into six outside wells, separately, and then precipitation lines were observed after 24 and 48 hours incubation at 37°C . As the results, almost all the same precipitation lines were observed in cases of both antigens.

For making antigen of the FA test, cysts were taken out from the serous membrane of the intestine of rats infected with the Hawaii strain or the NIAH strain. After cysts were crushed in a small amount of PBS, a drop of bradyziote suspension was made smear on a slide glass, air-dried, fixed with acetone and used as antigen. In the FA test, both antigens were stained positive by the same dilution (1:10) of both FA conjugates each other, whereas *Toxoplasma* antigen as control was negative. When each antigen was absorbed with heterologous positive serum, both antigens were not stained by both FA conjugates. Thus, the Hawaii strain was found to be not different from the NIAH strain in the agar gel diffusion and FA tests.

Cross immunity of both protozoa was examined in cats. The cats had fed cysts of the NIAH strain were challenged with cysts of the Hawaii strain after finishing the oocyst discharge. The cats discharged a smaller number of oocysts for a shorter period than control cat which received cysts of the latter alone, as shown in Figure 1.

On the contrary, when cats were primarily infected with the Hawaii strain and challenged with the NIAH strain, similar pattern was observed in their oocyst discharge. Such a similar pattern in oocyst discharge was also observed when only the NIAH strain was used in both primary infection and challenge.

Therefore, the differences between *I. bigemina* large type of cats and *B. wallacei* are no longer recognized. The former was also identified the same species serologically and immunologically as the latter. Then, *I. bigemina* large type of the cat will be designated as the NIAH strain of *B. wallacei*, hitherto.

Table 1. Infectivity to hamsters of the Hawaii and NIAH strains

Strain	Examination of cysts	Oocyst discharge of infected cats ^{a)}
Hawaii	0/17(0/9) ^{b)}	2/8(2/4)
NIAH	0/9	2/5

a) Fed organs of the infected hamster.

b) Number positive/Number examined or inoculated.

In parenthesis the second examination only.

Fig. 1. OPG in kittens A (●—●) and B (×—×) challenged with the Hawaii strain finishing the oocyst discharge in the primary infection with the NIAH strain and kitten C in control group (▲—▲).

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要 約

猫由来 *Isospora bigemina* 大型種と *Besnoitia wallacei* の比較試験 (短報): 伊藤進午・志村亀夫 (農林水産省家畜衛生試験場)——*Besnoitia wallacei* のオーシスと猫由来 *Isospora bigemina* 大型種との比較を行った。前者のハムスターへの感染性は軽度ながら認められ、また感染猫腸内のシゾントの大きさも後者と差がなかった。さらに両者は血清学的にも免疫学的にも一致した。