Susceptibility of Djungarian Hamsters (Phodopus sungorus) to Neospora caninum Infection

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Neospora caninum is a Toxoplasma-like protozoon first identified by Dubey et al. in 1988 [4]. A wide range of mammals including cows, sheep, goats, horses [5] and dogs [2, 4, 5] were infected with N. caninum. Dogs, cats, mice, rats [5], and some species of gerbils (Meriones unguiculatus, Meriones tristrami, and Psammomys obesus) [2, 6, 10] were experimentally infected with the parasite. N. caninum tachyzoites cause a fatal neuromuscular disease in dogs, and develops abortion and stillbirth in cattle. In 1998, dogs were confirmed to be the definitive host of the parasite [9], however, the complete life cycle is not yet been clarified. The known modes of N. caninum infection are transplacental from a dam to her fetus [5] and by the ingestion of oocysts originated from the feces of dogs [3]. As only a part of transmission mode and life cycle of the parasite is known, it is indispensable to get some rodents experimentally susceptible to N. caninum infection for the fundamental research of this parasite. Since many species of rodents are comparatively easy to handle, they have been used to analyze the infection mechanism of N. caninum. Immunosuppressed or immunodeficient mice treated with methylprednisolone acetate and others and athymic nude mice were reported to develop severe clinical signs and often died of N. caninum infection, but this parasite is not pathogenic for outbred mice [5]. Thus, no satisfactory rodent model with normal immune function has been reported except for some species of gerbils [6, 10]. Until now, several species of hamsters have been reported susceptible to some parasites. For example, Syrian hamsters are susceptible to Babesia microti [1] and Leishmania donovani [7], and Chinese hamsters are to Acanthamoeba keratitis [11]. Considering these facts, we examined, as a first step, Djungarian hamsters (Phodopus sungorus) for the susceptibility to N. caninum tachyzoite infection because they were easily available for us. Animals used were 29 8 to 12-week-old conventional Djungarian hamsters bred in our laboratory. N. caninum tachyzoites of a Japanese isolate JPA1 [12] were cultured in Vero cell monolayers. The cultures were maintained in the minimum essential medium (MEM; Gibco-BRL, Gaithersburg, MD, U.S.A.) supplemented with 5% fetal calf serum (Harlan Sera-Lab, U.K.) and 1% MEM vitamin solution (Gibco-BRL). Prior to inoculation into Djungarian hamsters, N. caninum tachyzoites were harvested from cell monolayers by mechanical disruption and counted with a hemocytometer. Twenty-nine Djungarian hamsters were intraperitoneally inoculated with 5 \times 10^6 N. caninum tachyzoites. These hamsters were killed 3, 4, 5 and 9 weeks post inoculation (PI). Brains were removed from all the animals and crushed with a cover glass, and then tissue cysts were counted in the aliquot. Various visceral organs removed from the animals with clinical signs were fixed in a 10% neutral buffered formalin solution, and embedded in paraffin for histopathologic examinations. Paraffin-embedded samples were sectioned, and the sections were stained with hematoxylin and eosin (H&E) and examined microscopically.

Clinical signs, such as roughened coat, anorexia, emaciation and ataxia, were observed in 3 Djungarian hamsters inoculated with N. caninum tachyzoites. Of these hamsters, one died 9 days PI, but did not necropsied. The other two were killed 16 days PI and examined histopathologically. Histopathologic examination, revealed that many tissue cysts were observed in the cerebrum of both animals (Fig. 1a), and a cyst was found in the muscular tunics of stomach in one of the two hamsters (Fig. 1b). Non-purulent inflammation was found around cysts. Tissue cysts were also observed in the brain squashed of 26 out of 29 Djungarian hamsters inoculated with N. caninum tachyzoites (Table 1, Fig. 1c). The number of tissue cysts in brains ranged from 3 to 260 within 5 weeks PI, but more than 100 cysts were found in all the hamsters after 5 weeks PI.

Neospora caninum was described by Dubey et al. in 1988 [4], and the only known mode of transmission in the field are transplacental [5] and oral [3]. It would be necessary to get rodents susceptible to N. caninum infection. To obtain cysts of the parasite stably from the model rodents will help
to elucidate the infection mechanism by oral inoculation with them. These animals are practically useful to isolate *Neospora* organisms from naturally infected animals and to maintain them in vivo. Until now, the only rodents, which are susceptible to *N. caninum* infection and have a normal immune system, are some species of gerbils [6, 10]. It is important to search for non-immunosuppressed susceptible model rodents containing some species of gerbils in order to expand the researches on neosporosis. Since a few species of hamster had been reported to be susceptible to some parasites [1, 7, 11], we speculated that hamsters would be susceptible to *N. caninum* infection in addition to some species of gerbils. In the present study, we examined Djungarian hamsters for the susceptibility to *N. caninum* infection. In this study, *N. caninum* cysts were observed in the brain of 26 out of 29 Djungarian hamsters which were intraperitoneally inoculated with $5 \times 10^6$ tachyzoites of *N. caninum* JPA1 strain, and three hamsters showed clinical signs. Five weeks after inoculation, the cyst was formed in the brain of all the Djungarian hamsters inoculated and the number of *N. caninum* cysts detected was more than 100 per animal. Consequently, both results indicate that the Djungarian hamster is suitable for the studies on neosporosis. However, *N. caninum* cysts would be missed in the brain of 3 hamsters until 4 weeks PI. Therefore, the infection with *N. caninum* should be determined in Djungarian hamsters after 5 weeks PI. Djungarian hamsters with normal immune system showed clinical signs such as ataxia, sometimes died, and produced tissue cysts in the brain against the infection with *N. caninum* tachyzoites as well as some species of gerbils [8, 10]. Further, these hamsters are easily available handled and bred, so using Djungarian hamsters, various experiments, such as a protection test against *N. caninum* infection with the antigen and an elucidation study of the transmission route of the parasite, will become easily conducted. The infectivity of cysts (bradyzoites) and oocysts to Djungarian hamsters is needed to be studied in the future.

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