

**NOTE**  Parasitology

**Neospora caninum** Infected the Alimentary Tract of Nude Mice and was Transmitted to Other Mice by Intraperitoneal Inoculation with the Intestinal Contents

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(Received 30 August 1999/Accepted 7 December 1999)

**ABSTRACT.** *Neospora caninum* (BT-2 strain) that originated from the brain of a Holstein calf was serially passaged through 10 generations of BALB/c nude mice by intraperitoneal inoculation. Histological examination of the mice revealed that numerous clusters of tachyzoites appeared in the pancreas, stomach and small intestine as well as in the central nervous system (CNS) and skeletal muscles. Intestinal contents of the infected mice were inoculated intraperitoneally into uninfected nude mice and 3 of the 17 inoculated mice showed clinical signs at post inoculation days 3 to 10. The present experiments demonstrated a proliferation of *N. caninum* tachyzoites in the mucosa of the alimentary tract and pancreas of the nude mice and the intestinal contents of the mice were infective to other nude mice. —**KEY WORDS:** intestinal content, *Neospora caninum*, nude mouse.

Cyst-forming *Neospora caninum* is an apicomplexa protozoan first reported in 1988 [8]. The parasite is especially important as a causative agent of abortion and neonatal mortality in cattle [1, 2, 19]. Cats and mice have also been infected experimentally and shown to develop clinical disease [11, 15–17]. Encephalomyelitis and polymyositis are the major lesions observed in the bovine fetuses and dogs [3, 5, 7, 9, 21]. *N. caninum* can be transplacentally transmitted in naturally and experimentally infected animals [4, 6, 10, 12–14, 19].

Recently, oocysts were detected in the feces of dogs fed with *N. caninum* tissue cysts of mice, and immunosuppressed mice inoculated subcutaneously with the oocysts developed neosporosis [18].

In the present study, we established serial *N. caninum* passage using nude mice and examined the possibility of transmitting the parasite by intraperitoneal inoculation with intestinal contents.

**Experiment 1:** A Holstein calf (22-day-old, male) naturally infected with *N. caninum* and showing a precollostral antibody titer of 3,200 against *N. caninum* was obtained in Fukushima Prefecture. Nonsuppurative encephalomyelitis with *Neospora* cysts and polymyositis were observed on histological examination after necropsy. The brain of the calf was homogenized in physiological saline solution containing antimicrobials (1,000 IU of penicillin G and 100 μg of streptomycin/ml of saline solution). Homogenized tissues were filtered through sterilized gauze and centrifuged at 350 × g for 10 min, and the supernatant was discarded. The sediment was suspended in saline solution and approximately 1 ml of the sediment per mouse was inoculated intraperitoneally into five nude mice (BALB/c A Jcl-nu; CLEA JAPAN Inc., Tokyo). The brains, skeletal muscles and livers of the infected mice were homogenized similarly to the above and passaged serially through 10 generations of three to five mice each. A total of 40 mice were used in this experiment and housed in a germ-free isolator at 23 ± 1 °C and 55 ± 7% humidity. The animals were fed with a radio sterilized diet (CLEA JAPAN Inc.) and autoclaved water. The infected mice were observed every other day and killed by ether inhalation when they showed severe clinical signs. The mice were necropsied at post inoculation days (PID) 15 to 125. Tissue samples collected at necropsy were fixed in 10% neutral buffered formalin, dehydrated through graded alcohols, embedded in paraffin-wax, and sectioned at 4 μm in thickness and stained with hematoxylin and eosin.

Immunohistochemical examination using anti-*N. caninum* goat serum (VMRD Inc., WS, U.S.A.) was undertaken to detect *N. caninum* organisms in the tissues of mice, and biotinylated rabbit anti-goat serum (Vector Laboratories, CA, U.S.A.) was used as the secondary antibody. Seventeen nude mice (2 to 3 mice for each inoculum) were inoculated intraperitoneally with approximately 1 ml of the filtrate. Three of the mice were killed at PID 3 to 10 by ether inhalation at the terminal stages of infection, and tissue samples were collected at necropsy and processed as in the Experiment 1. The remaining mice did not show any clinical signs until PID 76. To examine the presence of oocysts, the intestinal contents of ten of the mice infected with *N. caninum* in the Experiment 1 were diluted with 10 volumes of sucrose solution and the flotation supernatant was dropped on a slide and examined under a light microscope.

Five mice inoculated with the brain tissue of the calf showed clinical signs, including emaciation, torticollis and...
tetraplegia at PID 44 to 125. At the following passages also, the mice showed similar clinical signs at a mean of PID 22.9 ± 6.3. All the mice inoculated with N. caninum showed marked enlargement of the systemic lymph nodes, and inflammatory lesions with tachyzoites were found in the CNS, heart, skeletal muscles, pancreas, tongue, stomach and intestines. At the first passage, the heart, skeletal muscles, pancreas, stomach, intestine, tongue, and CNS developed mild histological changes from PID 44 to 125. After the second passage, the lesions were most prominent in the CNS, skeletal muscles, pancreas, and stomach. Lesions in the CNS were characterized by focal necrosis or vacuolation in both grey and white matters, accompanied by a mild to moderate infiltration with neutrophils, lymphocytes of various size, and macrophages around the necrotic foci. Clusters of tachyzoites were found inside these foci and occasionally scattered over the lesions. Generally, in the heart and skeletal muscles, necrosis was widely spread associated with inflammatory cell infiltration, but N. caninum tachyzoites were rarely seen in these lesions. The pancreas showed severe interstitial fibrosis, necrosis of exocrine cells and inflammatory cell infiltration in the interlobular ducts. Clusters of tachyzoites were occasionally found in the exocrine cells and around the dilated pancreatic ducts (Fig. 1). In the stomach, necrotic foci containing clusters of tachyzoites and infiltration with neutrophils, eosinophils, lymphocytes and macrophages were scattered throughout the mucosa and tunica muscularis (Figs. 2, 3). Similar lesions, though mild to moderate, were observed in the mucosa of the small and large intestines. N. caninum tachyzoites were frequently found in epithelial cells of the salivary glands, skeletal muscles and peripheral nerves of the tongue. Other organs such as the liver, spleen, lungs and kidneys showed no significant histological changes throughout the experiment, except for hyperplasia of the lymphoid follicles in the spleen and lymph nodes of all the mice. N. caninum tachyzoites were found in six of the 17 mice inoculated intraperitoneally with the intestinal contents of three infected mice. Three recipient mice showed similar clinical signs to those observed in Experiment 1 at PID 3 (two mice) and 10 (one mouse), however, the remaining three mice did not show any clinical signs until 76 days. Histological examination demonstrated inflammatory foci with N. caninum tachyzoites in various organs of all the mice: mild to moderate encephalitis, pancreatitis, polynymositis, and gastritis. Mild inflammatory lesions were sparsely distributed also in the liver, lungs, spleen, and adrenal glands. No oocysts were detected in the intestinal contents of the infected mice by the sugar flotation method. In the present experiments, N. caninum originated from a calf proliferated in the mucosa of the alimentary tract and pancreas of the experimentally infected nude mice as described in the recent paper [20]. The mice developed neosporosis after intraperitoneally inoculated with the intestinal contents of infected mice. Therefore, the present results suggest that under experimental conditions tachyzoites are excreted into the intestinal contents of N. caninum infected mice, and the tachyzoites are infective to other mice by intraperitoneal inoculation.

REFERENCES

Fig. 1. Pancreas; nude mice. Clusters of tachyzoites (arrow) in pancreatic acinar cells and infiltration with inflammatory cells in the interstitium at 21 PID. HE and Avidin-biotin-peroxidase complex method, Mayer’s hematoxylin counterstain. Bars=20 μm.

Fig. 2. Stomach; nude mice. Numerous tachyzoites (arrow) in epithelial cells and mild infiltration with neutrophils, lymphocytes, and macrophages in the mucosa at 17 PID. HE stain. Bar=20 μm.

Fig. 3. Stomach; nude mice. Numerous immunopositive intracellular tachyzoites (arrows) in the mucosa at 17 PID. Avidin-biotin-peroxidase complex method, Mayer’s hematoxylin counterstain. Bar=20 μm.