

Neosporosis in a Dog: The First Case Report in Japan

Takashi UMEMURA, Katsuhisa SHIRAKI, Takehito MORITA, Akinori SHIMADA, Makoto HARITANI¹⁾, Masaru KOBAYASHI¹⁾, and Shizuo YAMAGATA²⁾

Department of Veterinary Pathology, Faculty of Agriculture, Tottori University, Koyama-cho, Tottori 680, ¹⁾National Institute of Animal Health, 3-1-1 Kannondai, Tsukuba, Ibaraki 305, and ²⁾Yamagata Animal Hospital, 472-9 Hirotoni, Okayama 704, Japan

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A cyst-forming sporozoon associated with encephalomyelitis and myositis, or polyradiculoneuritis in young dogs had been reported in Scandinavia and the United States since 1984 [1-3, 13]. The parasite microscopically resembled *Toxoplasma gondii*, but was ultrastructurally and antigenically distinct [1-3, 13]. From 1988 to 1990, Dubey *et al.* [5, 6, 8, 9] and Lindsay *et al.* [14, 15] reported the isolation and propagation of the sporozoon in cell culture, experimental reproduction of the disease in pups by transplacental transmission, and immunohistochemical diagnosis of the disease using specific antibody. They named the sporozoon *Neospora caninum* [5]. Canine neosporosis has been found so far in France [4], England [7], Canada [4] and Australia [16] as well as the countries mentioned above. Present report briefly describes clinical and pathological findings of *N. caninum* infection in a pup. This is the first case report of neosporosis in Japan.

A 5-month-old, female Sheltie was presented to the clinic complaining of hind leg weakness. Hematology and serum biochemistry were unremarkable except increased values of creatine phosphokinase (1198 IU/L) and Ca (11.2 mg/dl). Serum antibody titer to *T. gondii* was normal (<40). The clinical signs progressed to tetraplegia with cervical weakness, loss of pupillary reflex, dyspnea, and paralysis of the tongue and masseter. The dog died 20 days after the referral.

Necropsy revealed a discoloration of skeletal muscles. Formalin-fixed tissue pieces of major organs and skeletal muscles from various parts of the body were sent to our Department for pathological diagnosis. The spinal nerve roots and cranial nerves were not collected. Significant histological changes were in the liver, central nervous system (CNS), and all the skeletal muscles examined. The liver showed multiple minute foci of hepatocytic necrosis and reticuloendothelial (RES) cell nodules. The nodules frequently contained a few to less than 10 tachyzoites. Each tachyzoite was ovoid or lunate in shape and up to 2.5 μm in axial length.

Glial nodules, foci of gliosis and softening were densely or sparsely distributed throughout the CNS. The lesions were preferably located in the submeningeal (Fig. 1a) and periventricular areas of the CNS. Perivascular cuffings of mononuclear cells were occasionally found in the meninx and nervous tissue. Glial-mesenchymal scars were formed in the large malacic foci of the cerebral and cerebellar cortices, and submeningeal white matter of the medulla

oblongata and spinal cord. In and around the lesions there were many, round or elongated tissue cysts containing scores of bradyzoites (Fig. 1b). Most of the cysts were up to 30 μm in diameter, and had 1- to 2- μm -thick cyst wall. They were in the neuropil, cytoplasm of macrophages, glial cells, or nerve cells, but rarely in the vascular endothelial cells. A small number of free tachyzoites were occasionally seen around some of the tissue cysts.

The skeletal muscles were collected from the face, neck, fore- and hind-legs, lumbar region, abdominal wall, and tongue. All the muscles contained variably-sized, ill-defined areas of myositis in which degenerative, necrotic and regenerative myofibers were intermingled with a small number of inflammatory cells comprising macrophages, lymphocytes, plasma cells and neutrophils (Fig. 2a). Hyalinized myocytes occasionally contained small to large cluster of tachyzoites in their cytoplasmic vacuoles (Fig. 2b). Free tachyzoites presumably released from the ruptured cytoplasmic vacuoles were sometimes seen in the myositic foci.

Minute foci of myocardial necrosis were scattered in the heart and a few tissue cysts with a definite cyst wall lodged within cardiomyocytes. Neither tissue cysts nor tachyzoites were found in the other organs and tissues including the spleen, in which extramedullary hematopoiesis and activation of RES cells were prominent.

An avidin-biotin-complex immunoperoxidase technique (Vector Laboratories, California, U.S.A.) was employed on paraffin sections of the brain. The following rabbit antisera were used as primary antibodies: anti-*N. caninum* (courtesy of Dr. J. P. Dubey, Livestock and Poultry Sciences Institute, Maryland, U.S.A.) [15], anti-*Sarcocystis cruzi* (courtesy of Dr. J. P. Dubey) [11], anti-*T. gondii* (courtesy of Dr. K. Shimura, Poultry Disease Research Station, National Institute of Animal Health, Gifu), and anti-*Hammondia hammondi* (courtesy of Dr. K. Shimura) [17]. Both tachyzoites and tissue cysts showed intensely positive reaction with antiserum against *N. caninum* (Fig. 3), whereas antisera against *T. gondii* and *H. hammondi* reacted weakly with the wall of tissue cysts. Antiserum against *S. cruzi* stained bradyzoites, but didn't stain cyst walls and tachyzoites.

Electron microscopical examination was done on the formalin-fixed brain (tissue cysts) and skeletal muscles (tachyzoites). The tissue cysts lacked inner septal structure and had finely granular cyst wall of 0.7 to 1.2 μm (4 tissue cysts were measured)-thickness. Average sizes of bradyzoites and tachyzoites were 3.4 \times 2.3 μm and 3.1 \times 1.7 μm , respectively. Tachyzoites frequently showed en-

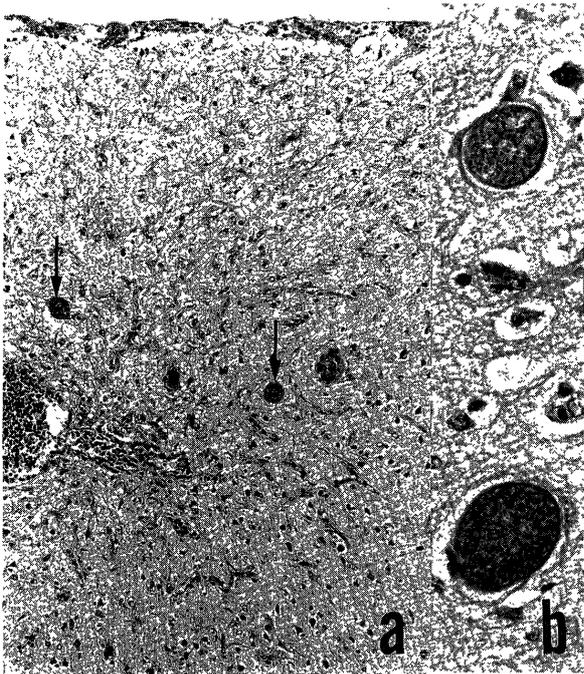


Fig. 1. a: Gliosis in the submeningeal cerebral cortex. Arrows indicate tissue cysts. $\times 88$. b: Higher magnification of tissue cysts in the cerebral cortex. $\times 180$.

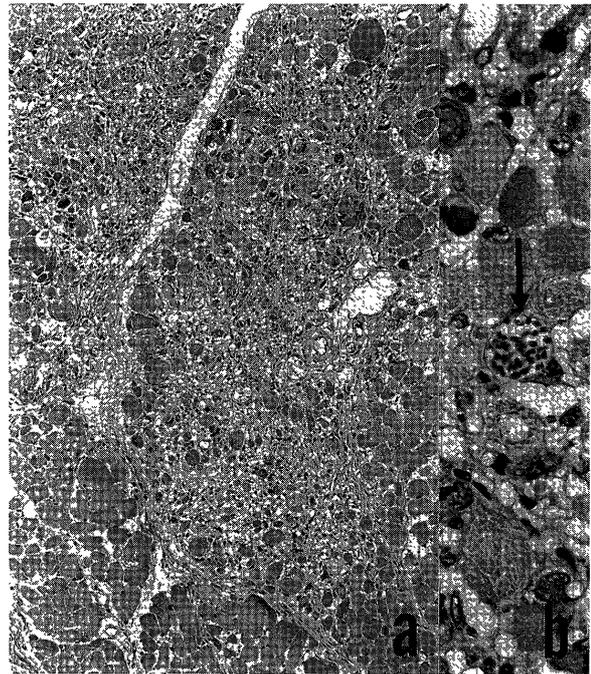


Fig. 2. a: Ill-defined focus of myonecrosis of the tongue. $\times 35$. b: Higher magnification of the skeletal muscle fiber containing many tachyzoites in the cytoplasm (arrow). $\times 450$.

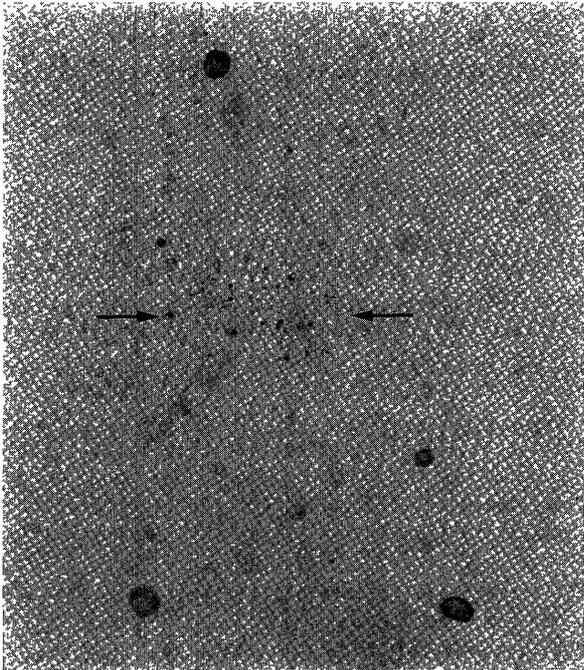


Fig. 3. Avidin-biotin-complex immunoperoxidase technique using primary antisera against *N. caninum*. Tissue cysts and tachyzoites (between arrows) in the cerebral cortex are positively stained. $\times 93$.

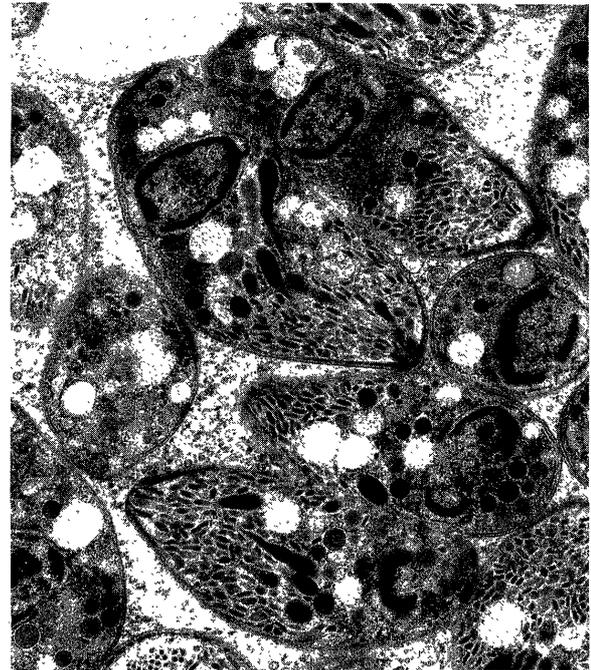


Fig. 4. An electron micrograph of tachyzoites in the cytoplasm of the skeletal muscle fiber. The tachyzoites show endodyogeny and the pellicles of daughter cell are partially fused. $\times 12,000$.

dodyogeny (Fig. 4) and many micronemes, some of which oriented perpendicular to the double-layered pellicle. The numbers of rhoptries were less than 10 in 40 tachyzoites ultrastructurally examined.

Canine neosporosis has been reported mainly in pups younger than one year old, but older dogs are also susceptible to the parasite [5]. Major clinical signs are hindlimb paralysis which rapidly progress to tetraplegia and death, and marked elevation of creatine phosphokinase [5, 12]. Histologically, necrotizing nonpurulent myositis of skeletal muscles and meningoencephalomyelitis are the most consistent findings [4, 8, 16, 19]. *Toxoplasma*-like organisms are seen in various organs including the skeletal muscles, CNS, and liver. A differentiation of *N. caninum* from other cyst-forming coccidia has been made based on ultrastructural findings (absence of septal structure in tissue cysts, thick cyst wall, frequent endodyogeny, numerous micronemes perpendicularly oriented to the pellicle, and many rhoptries in *N. caninum*) [4, 5, 18] and immunohistochemical results [15]. The present case coincided well with the reported cases of canine neosporosis in morphology and immunohistochemical reactivities of the protozoon as well as clinical and pathological characteristics.

N. caninum was found in the sediment of cerebrospinal fluid of the pup showing nervous signs [5]. This may account for submeningeal and periventricular distribution of the CNS lesions of the present case, in which the parasite was disseminated via cerebrospinal fluid.

Tissue cyst walls of *N. caninum* weakly reacted with antisera against *T. gondii* and *H. hammondi*, and bradyzoites within the cysts were positively stained with antiserum against *S. cruzi*. These results suggest a partial antigenic homology among the coccidia. The reactivities of these antisera with different components of the tissue cyst may be explained by the facts that the antiserum against *S. cruzi* was generated using saline extract of bradyzoites as antigen [11], and antisera against *T. gondii* and *H. hammondi* were derived from the rabbit immunized with oocysts of the coccidia (Dr. K. Shimura; personal communication).

Exact biology of *N. caninum* is yet to be clarified, because the life cycle, sources of infection, and host range

of the protozoon are unknown. The parasite has been experimentally transmitted to sheep [4], cats [10], dogs [8], mice [6], and rats [4]. Therefore, efforts are required to find neosporosis in animal and human cases formerly diagnosed as toxoplasmosis.

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