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Title	Development of diagnostic method for Neospora caninum and serological survey in cattle and humans
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against hepatocytic injuries due to cholestasis and play a major role in the hepatic fibrogenesis of chickens, same as in that of mammals.

There were no immunohistochemical differences between the Ito cells of the livers in broiler chickens affected with spontaneous cholangiohepatitis and livers with malformation of extrahepatic biliary tracts. Ito cells expressing HHF 35 and desmin actively prolifer-

ated in the fibrotic foci of the all livers.

The present studies demonstrated that extrahepatic biliary malformation with bile stasis sometimes cause cholangiohepatitis in chickens and that Ito cells play a major role of the hepatic fibrogenesis showing enhanced immunoreactivities in both spontaneous cholangiohepatitis and experimental cholestatic livers of broiler chickens.

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### Development of diagnostic method for *Neospora caninum* and serological survey in cattle and humans

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*Neospora (N.) caninum* is a recently recognized protozoa of animals, which had been misidentified as *Toxoplasma (T.) gondii* until 1988. *N. caninum* can infect cattle, dogs, rodents and other mammalian animals and the definitive hosts for this protozoa are dogs. Tachyzoites are found in many tissues of infected animals, but tissue cysts or bradyzoites are only found in the central nervous system. *N. caninum* is a major cause of abortion in cattle in many countries including Japan. It is suspected *N. caninum* is transmitted to cows vertically and horizontally.

The histopathological lesions of adult cows seropositive for *N. caninum* have not been previously described, and the parasite has never been isolated from the tissues of adult cattle. In this study, we isolated *N. caninum* from the brain of a 2-year-old dairy

cow that had aborted twice due to *N. caninum* infection. The cow was killed 24 days after the second abortion and the brain emulsion of the cow was inoculated to nude mice. Multifocal areas of perivascular cuffing and glial nodules were observed in the cerebrum and mesencephalon of the cow, and the brain lesions closely resembled those described in aborted fetuses due to *N. caninum* infection. Moreover, *N. caninum* was isolated from the brain of the nude mice inoculated with brain emulsion of the cow. These results suggest that the brain is one of the possible sites for latent residence of the parasite in infected, but clinically normal cows. The brain may be the organ from which reactivated tachyzoites enter the bloodstream and infect a developing fetus.

To compare the genome of the new isolate strain (BT-3) with that of other 4 strains

previously isolated from aborted fetuses, we used PCR procedure to amplify a *N. caninum* internal transcribed spacer 1 (ITS 1) DNA fragment. A DNA fragment of expected size (279 bp) was amplified from all strains, but no DNA fragments were amplified from a DNA extract of *T. gondii*. These results proved that BT-3 and other 4 isolates are *N. caninum*, and genomic differences for ITS 1

were not found among the isolates. Immunological reactivities of the 5 isolates of *N. caninum* with different sera from seropositive dogs, cattle and mice were investigated. No immunological differences were observed among the 5 isolates. Therefore, any one of these isolates can be used as antigen for the indirect fluorescent antibody test.

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### *In-vitro* culture of mouse preantral follicles and its application to the analysis of transforming growth factor- $\beta$ 1 expression in the developing follicles

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This study was conducted to determine the normality of cultured follicles including the developmental competence of their oocytes after *in-vitro* culture and to apply the culture system to the analysis of transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) expression in the developing follicles in mice.

First, mouse follicles isolated from ovaries were classified into two categories (types 4 and 5a) and were cultured *in vitro*. Oocytes derived from both types of *in-vitro* grown follicles had similar maturation (70 and 62%) and cleavage (30 and 35%) rates. Blastocysts were, however, only obtained from the oocytes derived from the type 5a follicles.

The second study was designed to determine the expression of TGF- $\beta$ 1 in mouse follicles grown *in vivo* by immunohistochemical staining of ovarian serial sections. Positive TGF- $\beta$ 1 staining in the granulosa and theca

cells was initially observed immediately before the time of antrum formation (type 5b follicles). The proportion of follicles with positively stained granulosa cells increased with follicular development from early antral (type 6) to preovulatory (type 8) stages (13 to 82%). The proportion of follicles with positively stained theca cells did not show a clear trend (~30%) before ovulation, but increased in retarded type 7 (79%) and unovulated type 8 (55%) follicles during the ovulation period.

The third study was conducted to determine the relationship among growth, steroid production and immunolocalization of TGF- $\beta$ 1 *in vitro* using preantral follicles of types 5a and 4 as models for normally developing and retarded follicles, respectively. At the time of antrum formation, follicular diameters were similar between the follicles originated from both types; however, antral follicles from the