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understand the catabolic activity of joint cartilage, so that keratan sulphate could be an noninvasive and possibly direct informative marker to detect cartilage pathology in horses with joint diseases.


Pathomorphological studies of cholangiohepatitis in broiler chickens

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Enlarged, discolored and firm livers, often associated with cholecystitis and extrahepatic bile duct distended with bile, have been reported in broiler chickens. Histopathologically, these livers showed a diffuse fibrosis with marked proliferation of biliary ductules, cholestasis, accompanied by heterophilic and lymphocytic infiltration. Although Clostridium perfringens (C. perfringens) has been suggested as the causative agent, the precise pathogenesis of the hepatic lesions remains unclear. In the present study, the relationship between cholangiohepatitis and malformation of extrahepatic bile ducts, pathomorphologic features of Ito cells in livers treated with extrahepatic bile duct ligation (BDL) and in livers of field cases were investigated in broiler chickens. The role of Ito cells in the hepatic fibrogenesis in these hepatic lesions was also discussed.

Six of the 88 abnormal livers from a slaughterhouse demonstrated biliary atresia caused by malformation of extrahepatic biliary tracts. Histologically, these livers consisted of diffuse fibrosis, diffuse proliferation of biliary ductules and distention of bile ducts, bile plugs, multifocal aggregates of heterophils, small foci of coagulative and lytic necrosis. Although C. perfringens type A enterotoxin was detected in three livers by immunohistochemistry, the inflammatory reaction to these bacteria was minimal. The hepatic changes were extremely similar to those experimentally induced by extrahepatic BDL, and suggested that some cases of cholangiohepatitis are caused by biliary malformation with bile stasis.

Experimental extrahepatic BDL in broiler chickens were performed in order to clarify the pathomorphological of Ito cells in hepatic fibrosis. The treated livers were enlarged with irregular surfaces, mildly consolidated and discolored to pale green. Immunohistochemistry demonstrated that Ito cells in normal livers expressed HHF 35-specific muscle actin, vimentin, desmin, glial fibrillary acidic protein (GFAP) and cytokeratin. In treated livers, HHF 35-and desmin-positive Ito cells were frequently found in fibrotic areas and were larger in size with more extensive immunoreactivities. Ultrastructural findings suggested that Ito cells actively react
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 HHF35 and desmin actively proliferated 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.


**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...