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INFORMATION

Hokkaido University conferred the degree of Doctor of Philosophy (Ph. D) in Veterinary Medicine on June 30, 2004 to 4 recipients.

The titles of their and other information are as follows :

Molecular Epizootiology of *Blastocystis* Infection in Japan

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Blastocystis hominis is a protozoan parasite in humans. *Blastocystis hominis*-like organisms have been found in various animals, which are indistinguishable from *B. hominis* morphologically. In this study, I carried out a survey of *Blastocystis* among various animals in western Japan, examined isolates from the animals molecularly, and analyzed the phylogenetic relationships between human *B. hominis* and the isolates from the animals.

Forty-one isolates from livestock and zoo animals were genetically analyzed to determine the genetic similarity among the isolates from humans and animals using diagnostic-PCR for subtype identification and PCR-RFLP of SSUrDNA for ribodeme identification, respectively. The result in the present comprehensive analysis strongly suggests that the nine isolates from cattle, pigs, and primates are closely related to human *B. hominis* and are designated to be ribodeme 1 or 2. However, the RFLP patterns of the six isolates from primates found to be variants of subtype 1 and of three isolates from birds found to be subtype 4 were slightly different from those of variant of subtype 1 (ribodeme 8) and subtype 4 (ribodeme 9) from humans, respectively. In the other 23 isolates from cattle, pigs, primates, and birds, neither subtype

nor ribodeme was not identified except three isolates from primates were designated as ribodeme 6. The SSUrDNA of nineteen *Blastocystis* isolates from animals was sequenced. Based on the results, the phylogenetic relationship among isolates from humans and animals was analyzed. The results suggested that the nineteen isolates were classified into 7 groups (I to VII) and that many of the isolates harboring in animals have zoonotic potential, or have cross-transmissibility among heterogeneous hosts. Correlations among "Subtypes", "Ribodemes" and "Group" have been clarified as follows: Group I consists of the isolates correspondent to Subtype 1 or Ribodeme 1; Group II to Ribodeme 6; Group III to Subtype 3 or Ribodeme 2; Group VI to Subtype 4; Group VII to Subtype 4; Group IV and V to unknown Subtypes and Ribodemes, respectively. The present study using various animal isolates revealed that some isolates were not applicable for the classification of subtypes or ribodemes, or for the correlation of subtypes and ribodemes proposed previously in human *B. hominis* isolates occurring in animals. This study also demonstrated that sequencing of SSUrDNA is superior to subtypes and ribodemes identifications for classification of genetically similar isolates.

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Epizootiological studies of zoonotic helminths of
red fox (*Vulpes vulpes*) and raccoon dog (*Nyctereutes procyonoides*)
in Hokkaido, Japan

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Red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) distribute throughout Hokkaido and serve as the main definitive host of *Echinococcus multilocularis*, one of the zoonotically important parasite. Recently, growing population of urban foxes has been observed in Hokkaido. There is a threat of environmental contamination with parasite eggs in urban residential area. This study was conducted to provide information on the epizootiological status of two zoonotic parasites, *E. multilocularis* and *Trichinella* species, in red foxes and raccoon dogs in Otaru city, Hokkaido.

Sixty-seven red foxes and 13 raccoon dogs were captured in the outskirts of Otaru City, and were examined for the parasites. Fifty-seven percent (38/67) of the foxes and 23% (3/13) of the raccoon dogs were found to be *E. multilocularis*-positive. The total biomass of the tapeworms from all infected foxes was 2,818,000, and the worm burden in each animal ranged from 1 to 550,000 (mean 74,000). The total biomass of the tapeworm from the 3 infected raccoon dogs was 1,500 worms. Ninety percent of the total biomass of the tapeworm were harbored by 24% (9/38) of the infected foxes.

The distribution of the tapeworm in the small intestine was determined. The middle part and the anterior part of the small intestine carried the highest worm burden in foxes and raccoon dogs, respectively. No significant differences in the prevalence were found between sex and age groups of foxes. Although the worm burden was significantly higher in juvenile than adult foxes, the fecal egg output in both age groups were not significantly different. In the present survey, gravid tapeworms were found in one of the raccoon dogs examined, and eggs were detected from feces of the animal.

The reliability of coproantigen-ELISA using monoclonal antibody, EmA9, for the diagnosis of *E. multilocularis* infection in foxes was confirmed by using the result of necropsy as a standard method. Foxes with 42 or less worm burden were coproantigen negative. There was a moderate correlation between the worm burden and the OD value. The sensitivity and specificity of the coproantigen-ELISA was 89.5% and 92.6%, respectively. While faecal egg examination showed comparatively low sensitivity. Usefulness of coproantigen-ELISA for the evaluation of the prevalence was confirmed in wild foxes.

In the present survey of *Trichinella* among 43 red foxes and 9 raccoon dogs, five foxes (12%) were found to be infected with muscle larvae of *Trichinella*. The mean number of larvae per gram of muscles was ranged from 7 in the lower jaw to 66 in the abdomen muscle. The larval burden in the diaphragm was comparatively higher than that in the other muscles. However, the mean larval density in the muscle of the diaphragm, shoulder, tongue tip, intercostal, tongue base and abdominal muscle (in increasing order) were higher than muscles of the other body sites.

Multiplex-PCR analysis using DNA from the muscle larvae from the foxes indicated that the parasite was *T. nativa*. This is the first record of *T. nativa* in Japan. The occupancy of this parasite in foxes with high prevalence was the first observation in Japan, and indicated the presence of an active sylvatic cycle of *Trichinella* in Hokkaido.

These results suggest that outskirts of Otaru City was an high endemic area of the two zoonotically important helminths, *E. multilocularis* and *T. nativa* species.

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Comparative pathological study of intestinal spirochetosis in herbivorous animals

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Some spirochetal species are cultivable, but abundant novel spirochetal species or phylotypes, which can not be presently cultivated *in vitro*, have been recognized. Intestinal spirochetosis has been reported in pigs and humans, but there is no report about the disease in herbivorous animals.

In Chapter I, a 24-month-old Holstein cow, which developed bloody diarrhea and weight loss, was examined. Invasive spirochetes caused enteritis in the cow. We designated this distinct entity "bovine dysentery", considering the etiology and pathology of the porcine counterpart, swine dysentery. In Chapter II, an epizootiologic survey with immunohistochemical methods was conducted for *Brachyspira* antigen-containing intestinal spirochetes in 27 diseased cattle in 19 farms in

Hokkaido, Japan between July 2000 and November 2003. The organisms were detected mainly in the large intestines of 10 cattle (37.0%) in 8 farms (42.1%). Other concomitant infectious agents were found in 80.0% of the cases with spirochetes, and Johne's disease was present in 50.0%. The spirochetes were considered to be moderately to slightly pathogenic and to aggravate erosive or ulcerative enteritis caused by some enteropathogenic organisms. The survey suggests that spirochetes are widely distributed in the cattle population. In Chapter III, to determine the relationship of intestinal spirochetosis to papillomatous digital dermatitis (PDD), 82 slaughtered cattle were examined. Concurrent affection was detected in 2 adult cattle, and morphologically indistinguishable spiro-

chetes showing invasiveness were observed in both lesions. Cattle with spirochetes in the intestine may be the most important carriers in terms of spread of PDD. Chapter IV deals with intestinal spirochetosis in deer. Of 7 deer, 6 had numerous *Brachyspira* antigen-containing spirochetes in the crypts of the cecum and colon. They were immunohistochemically and ultrastructurally similar to those in cattle. The bacteria would be a causative agent of enteritis, and their infection might be already prevalent among wild sika deer. In Chapter V, a 21-month-old Thoroughbred colt, showing continuous diarrhea and growth retardation for 7 months, was subjected to euthanasia for necropsy and laboratory examinations. *Brachyspira* antigen-containing spirochetes were morphologically

divided into 3 types. These invasive spirochetes might be enteropathogenic to horses. In Chapter VI, an epizootiologic survey with histologic, immunohistochemical and ultrastructural methods was conducted for *Brachyspira* antigen-containing intestinal spirochetes in 12 diseased or injured Thoroughbred horses, aged from 35 days to 17 years. The bacteria, which were found in 7 horses (58.3%), were more frequently seen in the cecum. Invasion of the mucosa by the spirochetes seemed to be the cause of epithelial hyperplasia in the cecum and colon.

The present studies on intestinal spirochetosis will increase awareness of its importance in herbivorous animals, and will be helpful for the diagnosis and treatment.

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Studies on ovarian follicular dynamics and fertilizability of oocytes in postpartum dairy cattle

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