Laboratory and Epidemiology Communications

Frequent Isolation of *Echinococcus multilocularis* from the Livers of Racehorses Slaughtered in Yamagata, Japan

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Communicated by Tomoyoshi Nozaki

(Accepted September 3, 2010)

An examination of the internal organs of a 5-year-old male thoroughbred horse brought to Yonezawa Municipal Slaughterhouse in Yamagata Prefecture on September 26, 2007 revealed over 20 white nodular hepatic lesions (diameter, 1–25 mm) (Fig. 1A). A full histopathological and genetic examination confirmed that the pathogen was *Echinococcus multilocularis* (Fig. 1B). This was the first time that this organism had been detected in a horse from a slaughterhouse outside Hokkaido, where the parasite is endemic. About 200 horses are brought to this slaughterhouse annually, all of which are observed by post mortem inspection at Yamagata Prefectural Nairiku Meat Inspection Center. Recent inspections have occasionally revealed a similar hepatic pathology to that found in the horse described in detail herein, which was diagnosed with alveolar echinococcosis. We examined the hepatic lesions in all horses brought to this slaughterhouse to determine the status of *E. multilocularis* infection.

A visual examination of the livers of 218 horses (217 thoroughbreds and one pony) brought to Yonezawa Municipal Slaughterhouse between October 31, 2007 and October 30, 2008 revealed nodular lesions in 78 of them. The areas with lesions were collected and bisected where possible. One part was fixed in 10% formalin and stored at room temperature for histopathological examination, whereas the other was frozen and stored at −20°C to genetically test for the pathogen. Sections stained with hematoxylin-eosin (HE) and periodic acid-Schiff (PAS) stain were assessed histologically. The white nodular lesions in 17 of the 78 horses were diagnosed as interstitial hepatitis, hepatic cysts, hepatic abscesses, or parasitic hepatitis accompanied by nematode sections, whereas those in the remaining 61 horses had granulomatous and fibrous tissue accompanied by the accumulation of lymphocytes, histiocytes, and eosinophils. Some nodules comprised a thick outer fibrous layer and a central necrotic area. A PAS-positive cuticle layer characteristic of *E. multilocularis* cysts was confirmed in 27 of the horses (Fig. 2). The cysts were empty and the walls comprised a thin cuticle lined by a germinal layer, although no brood capsules or protoscolices were detected in any of the lesions investigated.

About 25 mg of tissue was collected from the nodular lesions and its DNA was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The region of the mitochondrial 12s rRNA gene common to taenid tapeworms, including *E. multilocularis*, was amplified by PCR using the primer described by Dinkel et al. (1). PCR-RFLP was performed as described by Yagi et al. (2) to distinguish *E. multilocularis* from other taenid tapeworms. The PCR product was digested with the restriction enzyme *SspI* and then resolved by 10% PAGE. The characteristic cleavage pattern for *E. multilocularis* (175, 105, and 93 bp) was confirmed in 15 of the 27 horses with a histologically PAS-positive cuticle layer in the lesions, as well as in 14 of the 34 horses that lacked a cuticle layer. Six samples were selected at random from the PCR products of the positive samples and their nucleotide sequences found to match that of *E. multilocularis* originating in Hokkaido perfectly (EMBL/GenBank, AB024424).

The 27 horses with a PAS-positive cuticle layer in the histological samples and the 14 that were positive in the genetic test were all diagnosed with alveolar echinococcosis, thus leading to an infection rate of 18.8% (41 of 218). The diameter of the largest nodular hepatic lesion identified in the 41 infected horses was 14 mm. Lesions that were <5 mm in diameter were roughly spherical, whereas those that were ≥5 mm were irregularly shaped, ranging from crooked to bumpy spheres. The number of lesions ranged from 1 to >10, and most of the infected animals had lesions that were ≤5 mm in diameter. The original horse with hepatic lesions detected on September 26, 2007, which led to the present testing program, had >20 nodular lesions measuring 1–25 mm in diameter. The ages of the 8 male and 33 female horses that tested positive ranged from 4 to 9 years. We determined from interviews that 25 of the horses had been raised in Hokkaido, and that 16 had been bought and sold by several livestock dealers before being taken to the slaughterhouse, so we were unable to confirm where they had been reared.

Most reports of human or animal alveolar echinococcosis in Japan have originated in Hokkaido, where *E. multilocularis* is endemic. The final host in the sylvatic life cycle of *E. multilocularis* in Hokkaido is the red fox,
pigs can also serve as intermediate hosts in the life cycle of *E. multilocularis*. The first infected horse in Japan was discovered in Abashiri in eastern Hokkaido in 1983 (3). *E. multilocularis* infection in pigs, which was the world’s first reported natural infection by this organism, had already been detected in that area (4). Between 1983 and 2008, 30 of 19,957 horses tested in slaughterhouses across Hokkaido (average detection rate of 0.2%) were diagnosed with *E. multilocularis* infection (3,5) along with one racehorse from Hokkaido which died after an accident in 1991 (6). The present *E. multilocularis* infection rate of approximately 20% for horses tested in Yamagata is unusually high.

Statistics published by the Japan Race Horse Registry (2010) (7) show that 7,400–8,000 thoroughbred horses are produced annually in Japan, 95% of which are bred and reared in the Hidaka and Iburi regions of Hokkaido. The spread of *E. multilocularis* among animals has mirrored that of the habitat of infected foxes, which expanded from eastern Hokkaido to the whole island by around 1990, with the infection rate across Hokkaido increasing from 10–20% in 1990 to over 30% between 1995 and the present day (8). Taking into account how *E. multilocularis* has spread across Hokkaido, the high degree of infestation across the entire island since 1995 indicates that the environment of horses, such as pastures in the Hidaka and Iburi regions, has become contaminated with *E. multilocularis* eggs. Although we were unable to confirm that 16 of the 41 infected horses in the present survey were reared in Hokkaido, they were unquestionably connected with Hokkaido.

Development of the larval stage (metacestode) of *E. multilocularis* within a swine or equine host ceases at an early stage and the parasite is incapable of reproduction, thus meaning that development in these hosts does not play a role in completing the life cycle. However, the biological characteristics of *E. multilocularis* in these animals have not yet been elucidated. Most pigs are growing-finishing pigs that are usually slaughtered at approximately 6 months of age, which could explain why protoscoleces development has never been reported in any of the 30,000 pigs with known *E. multilocularis* infection in Hokkaido (5). However, whereas most pigs are slaughtered when young, horses can live for several decades after *E. multilocularis* infection. Thus, even though horses are generally unsuitable as intermediate hosts for larval *E. multilocularis*, the length of time involved and individual factors might result in protoscoleces formation. The main priority for preventing alveolar echinococcosis in Japan is therefore to prevent the disease from spreading outside Hokkaido, where it is endemic (9). Equine alveolar echinococcosis must be closely monitored and testing should proceed in slaughterhouses throughout Japan. In addition, horse livers and other organs containing *E. multilocularis* cysts must be properly disposed of to prevent infection of dogs and other hosts.

Acknowledgments We are deeply indebted to Drs. Kinpei Yagi (Hokkaido Institute of Public Health) and Takane Matsui (Obihiro University of Agriculture and Veterinary Medicine) for providing invaluable advice during the course of this study, and to Dr. Yoko Matsuzaki (Faculty of Medicine, Yamagata University) for DNA sequencing.

Conflict of interest None to declare.

REFERENCES