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Experimental Evaluation of Mink and *Apodemus speciosus* in the *Echinococcus multilocularis* Life-cycle in Hokkaido, Japan

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ABSTRACT: The epizootiological status of mink (*Mustela vison*) as definitive hosts and *Apodemus speciosus* as intermediate hosts in the transmission of *Echinococcus multilocularis* in Hokkaido, Japan, were evaluated by orally inoculating mink with protoscoleces, and *A. speciosus* with eggs of the cestode, respectively. No tapeworms were recovered from the alimentary tract of the mink, and no hydatid cysts were recovered from the viscera of the egg-inoculated *A. speciosus*. We conclude that mink and *A. speciosus* cannot serve as definitive hosts and intermediate hosts of *E. multilocularis*, respectively, in Hokkaido.

Key words: Echinococcus multilocularis, mink, *Mustela vison*, *Apodemus speciosus*, experimental infection, hydatid epidemiology.

The life-cycle of *Echinococcus multilocularis* in Hokkaido, Japan, is maintained primarily by foxes (*Vulpes vulpes schrencki*) and domestic dogs serving as definitive hosts, and Bedford's gray red-backed voles (*Clethrionomys rufocanus bedfordiae*) serving as intermediate hosts. Beside the two canids, spontaneous infection of the adult cestode has been reported from two domestic cats (Ambo et al., 1954; Yagi et al., 1984) and also from a raccoon dog (*Nyctereutes procyonoides albus*) (Yagi et al., 1988). Domestic cats experimentally infected with the Hokkaido strain of *E. multilocularis* have been quite refractory to the parasites as judged by the expulsion of most tapeworms from the gut before maturation; remaining cestodes were retarded in growth as indicated by slow segmentation (Kamiya et al., 1986).

Besides foxes and raccoon dogs, wildlife carnivores that inhabit the island of Hokkaido are brown bears (*Ursus arctos yesoensis*), sables (*Martes zibellina brachyura*), Japanese weasels (*Mustela itatsi*), feral American mink (*Mustela vison*), and feral raccoons (*Procyon lotor*). Uraguchi et al. (1987) reported that 25% of the food of feral mink in Hokkaido consisted of *Clethrionomys rufocanus bedfordiae*. However, to date, *E. multilocularis* infection in mink has not been reported. Although the susceptibility of *C. rutilus mikado* and *Apodemus argenteus* to natural infection by *E. multilocularis* in Hokkaido is well-documented, no such report regarding *A. speciosus* is available. Experimental infection of *A. speciosus* with *E. multilocularis* was attempted by Yamashita et al. (1958), using a single animal which was fed 20 eggs and necropsied on day 76 post-inoculation (PI). No metacestodes were observed. To clarify the epizootiological status of mink and *A. speciosus* in the transmission of *E. multilocularis* in Hokkaido, we experimentally infected both species with the cestode.

Five female 6-mo-old farm-ranched Pastel mink each were orally inoculated with 70,000 protoscoleces of the Hokkaido strain of *E. multilocularis*. The parasite used was isolated from a female *C. rufocanus bedfordiae* in Higashi-Mokoto, Hokkaido (43°48′N, 144°18′E) in May 1983 and has since been maintained in a Mongolian gerbil (*Meriones unguiculatus*) population in our laboratory. Two mink were killed 10 days PI and their digestive tracts examined for tapeworms. As a control, an adult dog was infected similarly with protoscoleces as were the mink.

To determine if *A. speciosus* can serve as an intermediate host in the transmission of *E. multilocularis* in Hokkaido, we orally inoculated four wild-caught *A. speciosus* with 300 eggs of *E. multilocularis* obtained from an experimentally infected dog on day 44 PI. To verify the viability of the eggs, three laboratory-reared *C. rufocanus bedfordiae* were similarly inoculated.

The recovery rate of the tapeworms from

the dog on day 10 PI was more than 35%. However, no tapeworms were recovered from the mink. A week later, the remaining three mink were reinoculated with 70,000 protoscoleces and examined for tapeworms 24 hr later. Again no tapeworms were detected in any mink. We concluded that mink cannot served as the definitive hosts of *E. multilocularis* in Hokkaido.

All rodents were necropsied on day 28 PI. No hydatid cysts were observed in the egg-inoculated *A. speciosus*, though two of the four animals each had a *Cladothyridium* sp. cyst in their livers. The three *C. rufocanus bedfordiae* had 22, 26 and 41 hydatid cysts in their livers, respectively. This experiment was repeated a month later using cortisone-treated *A. speciosus*. Three wild-caught *A. speciosus* each were injected subcutaneously with 2.5 mg cortisone acetate (Wako Pure Chemical, Osaka, Japan) six, four, and two days before oral inoculation with 300 eggs recovered on day 44 PI from the inoculated dog. Three laboratory-reared *C. rufocanus bedfordiae* were similarly inoculated with the eggs. Again, no hydatid cysts were observed in any of the cortisone-treated *A. speciosus*. However, 42, 45 and 52 hydatid cysts were recovered from the livers of the 3 *C. rufocanus bedfordiae*, respectively. Thus, *A. speciosus* appears to be refractory to *E. multilocularis* egg infection and cannot serve as its intermediate hosts in Hokkaido.

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