Studies on intermediate hosts of Echinococcus multilocularis in southwestern Montana
by Kathy Lee Eastman

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE
in Veterinary Science
Montana State University
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Abstract:
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multilocularis in southwestern Montana. Five species of potential intermediate hosts were examined:
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mice, Mus musculus; and 192 muskrats, Ondatra zibethicus. Two muskrats with E. multilocularis
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contained viable metacestodes which were used to induce artificial infections in 3 kittens. In 1970,
Leiby, Carney, and Woods (Jour; Parasit. 56, (#6): 1141-1150) reported infections with larval E.
multilocularis in two deer mice from eastern Montana. This report identifies another natural
intermediate host species in North America. Ecological implications of natural infections occurring in
muskrats are discussed.
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Date  May 26, 1978
STUDIES ON INTERMEDIATE HOSTS OF ECHINOCOCCUS
MULTILOCULARIS IN SOUTHWESTERN MONTANA

by

KATHY LEE EASTMAN

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Veterinary Science

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MONTANA STATE UNIVERSITY
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ABSTRACT

In 1976 a survey was initiated to determine the intermediate host(s) of the cestode *Echinococcus multilocularis* in southwestern Montana. Five species of potential intermediate hosts were examined: 118 deer mice, *Peromyscus maniculatus*; 16 shrews, *Sorex* spp.; 16 voles, *Microtus* spp.; 18 feral house mice, *Mus musculus*; and 192 muskrats, *Ondatra zibethicus*. Two muskrats with *E. multilocularis* liver cysts were found in Gallatin and Madison counties in December, 1977. This is the first confirmed natural infection in the muskrat with this larval cestode to be reported in North America. The cysts contained viable metacestodes which were used to induce artificial infections in 3 kittens. In 1970, Leiby, Carney, and Woods (Jour. Parasit. 56, (#6): 1141-1150) reported infections with larval *E. multilocularis* in two deer mice from eastern Montana. This report identifies another natural intermediate host species in North America. Ecological implications of natural infections occurring in muskrats are discussed.
INTRODUCTION

The causative agent of alveolar hydatid disease was first recognized by Virchow (1855) as being the larval stage of a cestode of the genus Echinococcus (34). The disease was believed to be restricted geographically to central Europe and parts of Russia for about a century following its discovery. In 1951, Rausch and Schiller mistakenly identified the etiologic agent of hydatid infection in microtine rodents as the larval form of Echinococcus granulosus, on St. Lawrence Island, Alaska (35). Preliminary studies showed microtine rodents to be the intermediate host of this cestode. Reindeer (Rangifer tarandus) were suspected as the intermediate host, but no infected reindeer were recovered. The occurrence of microtine rodents as intermediate hosts led to subsequent investigations of this parasite and human hydatid disease. In 1954, Rausch concluded that the larval cestode recovered in rodents was not E. granulosus, but rather a different species of Echinococcus. He based this conclusion on two characteristics of the infection by which it differed from E. granulosus: 1) the natural occurrence of the larval stage in microtine rodents; 2) an alveolar larval form which is produced through exogenous budding (36). He named the new species Echinococcus sibiricensis (36). Rausch alluded to the possibility this was the same parasite that caused alveolar hydatid disease in humans in Eurasia. When Rausch recognized E. sibiricensis
as a new species, research on this parasite was stimulated. The life cycle of the Eurasian cestode was worked out by Vogel in 1955, and found to be identical with *E. sibiricensis* (31). This cestode was subsequently identified as being conspecific with *E. multilocularis* Leuckart, 1863. Research during the next 20 years showed the widespread distribution of *E. multilocularis* and implications for human infections with alveolar hydatid disease. In 1956, Rausch reported the occurrence of this cestode on the mainland of Alaska (31). During a preliminary study in 1962, Fay and Williamson found *E. multilocularis* in arctic foxes (*Alopex lagopus*) on the Pribilof Islands (8). Choquette, MacPerson and Cousineau first reported *E. multilocularis* on the Canadian mainland (6). Subsequent studies in Canada by Hnatiuk (11,12), Baron (13), Chalmers and Barrett (5), Holmes (13), Lee (18), and Wobeser (44) showed this cestode occurs widely in Saskatchewan, Manitoba and Alberta. The occurrence of this parasite in the continental United States was first reported by Leiby and Olsen in North Dakota in 1964 (25). Since 1964, Leiby and coworkers have reported *Echinococcus multilocularis* in Minnesota (4), Iowa (20), and South Dakota (20). In 1970, Leiby, Carney and Woods recovered two naturally infected deer mice (*Peromyscus maniculatus*) from eastern Montana (20).

*E. multilocularis* is one of the most serious human parasites. Moreover, alveolar hydatid disease is often fatal, the symptoms and
—3—

disease resembling a carcinoma of the liver. Humans are a relatively
unsatisfactory host for the larva of *E. multilocularis*, for, as the
larval mass increases in size, the central portion dies and undergoes
degeneration and only the peripheral layer in close contact with the
involved tissue is able to survive (31). This parasite may also in­
vade the gall bladder, bile ducts, larger blood vessels and other
organs (31). In North America it has been reported in the arctic,
especially in Eskimo people. There are two main sources of infection
to these people: 1) Infected fox carcasses trapped for the fur trade
are brought into the camps to be skinned. 2) Ice is melted for water,
and fecal contamination of the ice occurs through the large number
of sled dogs maintained. Since alveolar hydatid disease is so
difficult to diagnose and medical doctors are poorly informed on this
disease, cases are probably overlooked in north-central America.
Leiby and Kritsky (21) reported infections in two adult house cats
from North Dakota. The implications for human infections in north­
central America with this parasite became much greater with the in­
volvement of domestic companion animals.

The 1971 report of infected deer mice in eastern Montana by
Leiby, Carney and Woods (loc. cit.) and subsequent reports of infected
red foxes (*Vulpes vulpes*) and coyotes (*Canis latrans*) throughout
Montana (41) illustrated the need for a more complete understanding
of the intermediate host(s) of this cestode in Montana. It was the
purpose of this study to survey potential intermediate hosts of *Echinococcus multilocularis* in southwestern Montana. Ecological implications of the distribution of this parasite will be discussed, including the public health significance of the distribution of *E. multilocularis* in rodents and possible life cycles in domestic animals which could result in human exposure.
MATERIALS AND METHODS

The Victor snap mouse trap was used to kill-trap 16 shrews, Sorex spp; 18 house mice, Mus musculus; and 118 deer mice, Peromyscus maniculatus. A few voles (Microtus spp.) were caught in kill snap traps, but most were collected by hand in the field. The rodents and insectivores collected during the summer of 1976 were trapped in areas where red foxes (Vulpes vulpes) infected with adult E. multilocularis were reported (40). Since all results were negative for summer 1976, during the summer of 1977, rodents and shrews were collected in close proximity to fox dens that had been active during the previous spring. Small mammals for this portion of the survey were all collected in Gallatin County, Montana. Most of these animals were collected in a flood plane biotype, which was grazed by domestic herbivores. A few of the voles were collected on irrigated farmland.

Muskrats (Ondatra zibethicus) which were collected for this survey in Gallatin, Madison, and Jefferson Counties were brought to the Montana State University Veterinary Research Laboratory by local trappers. Muskrat carcasses were labeled with date and site of collection. Each animal was assigned an identifying number and its sex was recorded at necropsy. All muskrats were trapped during the months of November and December 1976 and 1977.

At necropsy, potential intermediate hosts were assigned identifying numbers with weight and sex of each animal recorded, with the
exception that muskrats were not weighed. Those tissues with grossly visible lesions were fixed in 10% formalin. These specimens were sectioned at 6 μm and stained with hematoxylin and eosin for histopathologic examination. Tissue sections were examined with a Leitz Ortholux microscope to verify the presence of tapeworm larvae.

Metacestodes obtained from naturally infected muskrats were used to infect kittens (Felis catus) and via vegetative propagation were injected intraperitoneally into laboratory-reared deer mice, mongolian gerbils (Meriones unguiculatus) and golden hamsters (Mesocricetus auratus). Cysts were dissected from the liver and placed in saline solution. To separate the protoscolices from the rest of the cyst material, the cysts were ground with a pestle over a No. 50 mesh screen, and washed in 0.86% saline solution. The metacestodes were centrifuged and washed in saline three more times. The remaining material was then suspended in a known volume of saline, and numbers of scolices present were determined through an aliquot counting method.

Studies of intermediate host susceptibilities by Norman and Kagan (29) indicated gerbils to be a satisfactory experimental animal. Golden hamsters were shown to be refractory to the infection. Based on these results, two gerbils and two golden hamsters were purchased locally to test susceptibility of various rodent intermediate
hosts to intraperitoneal inoculation with *E. multilocularis* cyst material obtained from the two muskrats.

The two golden hamsters, two monogolian gerbils, and one laboratory-reared deer mouse were injected intraperitoneally with a .2 ml suspension containing 1,500 scoles removed from naturally infected muskrat liver tissue. The animals were also given 2,000 units procaine penicillin G and 2.5 mg dihydrostreptomycin sulfate to suppress secondary bacterial infection. Three more deer mice were each given 3,200 metacestodes intraperitoneally and an antibiotic combination as above. One other deer mouse was given a .6 ml injection containing 4,680 metacestodes intraperitoneally and 2,000 units procaine penicillin G and 2.5 mg dihydrostreptomycin sulfate.

At 88 days postinfection, one hamster, one gerbil and two deer mice which had received a .4 ml dose were killed by cervical dislocation and examined macroscopically for evidence of larval infection. The remaining animals were killed by cervical dislocation 113 days postinfection and examined for lesions. Suspected lesions were placed in 10% formalin. The pathology laboratory at the Veterinary Research Laboratory prepared slides of sectioned material for microscopic examination.

Cats were chosen to act as the experimental definitive host of *E. multilocularis*, based on two factors:
1. Cats have been shown to be a definitive host for this cestode both naturally (15,34) and experimentally (34).

2. The facilities were available to maintain experimentally infected cats in isolation.

Kittens were fed a known number of metacestodes mixed in canned catfood. They were maintained in the animal isolation unit at the Veterinary Research Laboratory. The kittens were given an initial dose of 6,240 scolices in 4 ml of solution. A second dosage of liver cyst material from the same muskrat was given the next day. This inoculum was freshly removed from the cyst and contained 46,800 scolices in a 6 ml suspension. The third dose, consisting of 18,000 scolices in a 5 ml suspension was freshly prepared from a second naturally infected muskrat liver cyst. Forty-six days after the third larval dose the kittens were euthanatized. The small intestine was opened longitudinally and washed and scraped over a 60 mesh screen to remove debris, and the washed contents were backed-washed through a 100 mesh screen. The remaining gut contents were examined macroscopically for evidence of adult Echinococcus multilocularis.
RESULTS

Results concerning the survey work and experimental infections are presented separately.

Survey Results

The life cycle of *Echinococcus multilocularis* is presented in Fig. 1, which shows possible involvement of domestic animals. Collecting of small mammals was initiated based upon the intermediate hosts implicated in this life cycle. Table 1 is a summary of the numbers of small mammals trapped. Previously, recovery of larval *E. multilocularis* within the state has been reported only in deer mice in eastern Montana (20). In this survey two muskrats with *E. multilocularis* liver cysts were found in Gallatin and Madison Counties in December, 1977. This is the first confirmed natural infection with this larval cestode in the muskrat to be reported in North America.

In Gallatin County, small mammals were trapped in locations where foxes harboring the adult cestode had been trapped. The map in Fig. 2 correlates collecting areas for rodents (other than muskrats) and insectivores with sites where positive foxes were collected. The map in Fig. 3 shows collecting locations for muskrats. One adult female muskrat positive for larval *E. multilocularis* was trapped in the East Gallatin River, where 16 muskrats were collected in 1977. The portion of the East Gallatin and Gallatin Rivers between the towns of Belgrade and Three Forks is located to the northwest of
Fig. 1. Life cycle of *Echinococcus multilocularis* with domestic animal involvement and possible human infections (15).
### TABLE I

Summary of the numbers of small mammals examined for larval *Echinococcus multilocularis*.

<table>
<thead>
<tr>
<th>Animals Examined</th>
<th>Number infected</th>
<th>Number examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deer Mice</td>
<td>0</td>
<td>118</td>
</tr>
<tr>
<td><em>(Peromyscus maniculatus)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shrews</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td><em>(Sorex spp.)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voles</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td><em>(Microtus spp.)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>House mice</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td><em>(Mus musculus)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muskrats</td>
<td>2</td>
<td>192</td>
</tr>
<tr>
<td><em>(Ondatra zibethicus)</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig 2: Collecting areas for rodents (other than muskrats) and insectivores correlated with sites where foxes positive for *E. multilocularis* were collected.
Fig. 3. Trapping sites for muskrats.
Bozeman. Of thirteen muskrats trapped along the Madison River between Norris and Three Forks in 1977, one adult male was positive.

The photomacrograph in Fig. 4 shows the liver of a muskrat naturally infected with larval E. multilocularis. The photomicrograph in Fig. 5 shows sectioned cyst material from a naturally infected muskrat. Several sections of liver were examined for histopathologic changes in the liver tissue, and for observations on cyst structure. The hepatic parenchyma was displaced and compressed into a narrow band at the periphery of a large, irregular, multilocular cyst. The loculi were of variable size and shape and were surrounded and separated by a thin fibrous capsule. These loculi were lined by a thin parasite membrane from which numerous scolices were noted to bud (brood capsules). Large numbers of calcareous bodies were noted within these brood capsules. A few daughter cysts were noted to invade the hepatic parenchyma adjacent to the large cyst. Inflammatory changes in surrounding tissue were minimal in these sections.

Experimental Infection Results

The successful experimental infection of three kittens with larval cyst material from the two naturally infected muskrats showed the metacestodes to be viable. The kittens harbored 182, 151, and 1074 adult E. multilocularis respectively in the small intestine at necropsy 46 to 50 days after inoculation. The photomicrograph in
Fig. 4. Photomacrograph of a muskrat liver naturally infected with larval *E. multilocularis*; approximately X3.
Fig. 5. Sectioned cyst material from a muskrat naturally infected with larval E. multilocularis; approximately X9.
Fig. 7 illustrates an adult cestode recovered from one of the kittens. Adult specimens reared in kittens were between 1 and 2 mm long and in general conformed to the description presented by Wardle, McLeod and Radinovsky (44). There were gravid proglottids present in a few of the specimens.

Attempts to infect deer mice, gerbils and hamsters experimentally by intraperitoneal injection of protoscolices were not successful. However, one hamster did develop a sterile cyst in the lower abdomen.
Fig. 6. Protoscolex from a muskrat liver naturally infected with larval *E. multilocularis*; approximately X1600.

Fig. 7. Adult *E. multilocularis* recovered from an experimentally infected kitten; approximately X100.
DISCUSSION

The recovery of *Echinococcus multilocularis* in the intermediate host(s) in southwestern Montana differs from other areas where this parasite is established. Leiby, Lubinsky, and Galaugher (23) reported a 15% prevalence in deer mice in a field collection of 99 in Manitoba. In North Dakota, Leiby (19) recovered 3 positive deer mice from a collection of 15. In the present field survey, 118 deer mice were examined, all of which were negative. Leiby and Kritsky (22) found strip mine areas in early successional stages of revegetation to be more favorable for maintaining high population densities of deer mice. The fox-deer mouse link would be a major pathway in the food chain. Southwestern Montana is composed mainly of mountainous and flood plane biotypes in which the habitats surveyed may support populations of a variety of species of rodent intermediate hosts. As a result the fox-rodent portion of the food chain probably is only one link of numerous alternate pathways as contrasted with enzootic areas in North Dakota where fewer alternative intermediate hosts are available. Leiby and Kritsky (loc. cit.) concluded that the prevalence of larval *E. multilocularis* is greatly influenced by the interdependence of the final and intermediate hosts. Kennedy (16) stated that host-parasite systems are unstable and helminth infection levels depend upon local host behavior and habitat.
Since the relationships between final and intermediate host vary and are interdependent, it is important to examine the food habits and predatory relationships of the hosts involved. The majority of studies have shown that foxes prefer small rodents, rabbits, wild fruits, berries and insects. They are opportunistic feeders and take any acceptable food in proportion to its availability (1). In a comprehensive study of fox predator-prey relationships in Iowa, Scott (39) found foxes preyed most on cottontail rabbits and would feed on deer mice whenever other food items were not available. During one summer of Scott's survey period no deer mice were eaten, and foxes were preying heavily upon muskrats during a drought. To correlate this information with conditions present during the summers of the present survey, data on streamflow from the United States Department of Agriculture, Soil Conservation Service were checked (42,43).

Streamflow during April through September 1976 was above average in the Gallatin and Madison Rivers. The April through September 1977 streamflow was one of the lowest ever recorded, with streamflow being as low as 40% of average in these rivers. Irrigation further accentuated the reduced streamflow. The natural barriers between muskrats and canine or feline predators were probably reduced during 1977 as muskrats were depending upon terrestrial vegetation for food.

It is my hypothesis that during periods of reduced streamflow in 1977 muskrats in southwestern Montana were stressed, forced
onto the land and subjected to heavy predation by foxes. The muskrats became infected by accidently ingesting infective ova while foraging on land.

Although *Echinococcus multilocularis* occurs throughout a wide geographic range, the distribution of this parasite tends to be focal in nature. It becomes established in areas where food-chain relationships necessary for its survival exist. Natural infections have not been reported in muskrats in North America, although experimental infections in muskrats have been successful (30). Therefore, the recovery of 2 muskrats naturally infected with larval *E. multilocularis* does not indicate this rodent is the principle intermediate host, but it suggests that under certain environmental conditions muskrats may become an important host. The habitat and variety of rodent species in southwestern Montana may favor a number of intermediate-final host patterns.

In host-parasite systems such as *E. multilocularis*, transfer to the next host can be achieved only by ingestion of the intermediate host. Death of the host is essential, and provided that the parasite doesn't kill the host before this, it is of no particular advantage to the parasite to avoid damaging the intermediate host (16). The large proliferating cysts of this cestode are highly pathogenic to
the intermediate host, and may even render it more susceptible to predation. Muskrats environmentally stressed and clinically affected with echinococcosis would be an easy target for predators, possibly even including smaller carnivores which aren't normally capable of killing muskrats such as domestic cats and smaller dogs.

The experimental infections done in conjunction with the field survey had a two-fold purpose. It was the primary purpose of the experimental infections to confirm the diagnosis of larval *E. multilocularis* in two naturally infected muskrats. A secondary purpose was to test susceptibilities of various intermediate hosts.

Kagan, Norman, and Leiby, (15) isolated adult cestodes from naturally infected foxes and experimentally infected cotton rats (*Sigmodon hispidus*) and mongolian gerbils. They considered typical infections of *E. multilocularis* to be biological verification of the identity of this parasite. Obtaining positive infections with the adult cestode in all three kittens experimentally infected confirms the diagnosis of this larval cyst occurring naturally in muskrats. The 100% infection rate also confirms the ability of the Montana strain of *Echinococcus multilocularis* to infect domestic cats. This could be an important route of human exposure in urban areas and on farms where cats often forage on small mammals.

During 1956-57, Sadun, Norman, and King (38) tested the susceptibility of various rodents to infections with larval *Echinococcus*
multilocularis in an attempt to find suitable laboratory animals for routine infections. They found the cotton rat to be most susceptible of nine species which they inoculated with this parasite. They were also able to infect 4 of 6 cotton mice (Peromyscus gossypinus) and 2 of 6 deer mice. An attempt to infect 3 golden hamsters was not successful. Other attempts to infect hamsters have not been successful (29).

The results of experimental injections with viable protoscolices differed from other reported results. Both gerbils were negative, and although one hamster showed no lesions, the other hamster had a sterile cyst in the lower abdomen. In rodents experimentally inoculated by this technique, cysts are frequently free from host tissue or only attached superficially (29). The free cyst in the lower abdomen was not unusual, however the fact it grew in a hamster was noteworthy.

Results of feeding experiments showed the vole to be more susceptible than the deer mouse to the North Dakota isolate of E. multilocularis (34). However, Kritsky and Leiby (17) observed higher infection rates in deer mice than in voles, which they attributed to the omnivorous feeding habits of deer mice and their tendency to inhabit areas in or near carnivore dens. The vole generally occurs in highly vegetated areas, with vegetation utilized for food as their only likely source of exposure (17). Ohbayashi, Rausch,
and Fay (30) found only 2 of 10 deer mice positive for larval *E. multilocularis* in experimental inoculations with viable protoscolices. During the course of this study, attempts to infect six deer mice failed. Since deer mice can be considered somewhat refactory to larval invasions, the negative results were not unusual.

Rausch and Richards (*loc. cit.*) observed differences in the ability of the larvae of *E. multilocularis* to develop in rodents of various species, although the cestode in central North America appeared to be indistinguishable from that found in the arctic. They felt such differences in the central North American strain represented adaptive changes that occurred in response to selective factors in a community of which this cestode has only comparatively recently become a part. The small series of experimental inoculations in this study can not be used to draw conclusions about susceptibilities of various intermediate hosts to the Montana isolate of *Echinococcus multilocularis*.

Virchow (1855) first investigated alveolar hydatid disease and reported that certain cases of malignancy of the liver were caused by the invasive growth of a larval cestode of the genus *Echinococcus* (31). The identity of this cestode remained controversial for about a century following its discovery. When Rausch recognized the new species, *Echinococcus sibiricensis*, he suggested the possibility of this being the same parasite that caused alveolar hydatid disease in humans (36). Research done by Rausch and others during the past
25 years showed the widespread distribution of *E. multilocularis* and implications for human infections. The larval invasion of this cestode produces alveolar hydatid disease, which is one of the most serious human parasitic diseases. Exposure to the infection in persons, as in rodents, is by ingestion of the eggs of the cestode through fecal contamination of food, water or other materials taken into the mouth (7). The degree of risk of exposure is therefore directly proportional to the abundance of eggs in the human environment and to the efficacy of mechanisms for fecal contamination of ingestible materials (7). The risk of human exposure relates to the biologic, sociologic conditions present in a given area and the life cycle in natural hosts.

Bozeman, located in southwestern Montana, is a city of 23,000 people. The agricultural areas surrounding Bozeman are characterized by irrigated farmland, pastures and mountains. Interspersed within the agricultural lands are areas of urban development. Small mammals, such as ground squirrels, muskrats, deer mice, and house mice live in and around these developed areas.

There are 2 main routes of human exposure in this area. Foxes and coyotes are trapped for their fur, resulting in exposure to infective ova from improper handling of these furs by trappers, fur buyers and others involved in processing of pelts. The second and probably most important route is through fecal contamination by
domestic companion animals. Wobester (45) examined 131 cats for
E. multilocularis and found 3 of the cats infected. One of these
cats was from a rural environment, the other two having been collected
as stray animals within the city of Saskatoon. Leiby and Kritsky (21)
reported two cats infected with this cestode and also recovered a
house mouse infected with larval E. multilocularis in the vicinity.
These results confirmed that under natural conditions both cats and
house mice established a predator-prey relationship necessary for the
maintenance of a domestic life-cycle of this parasite. In 1968, Leiby
and Nickel (24) found that ground beetles can ingest and transport
ova of E. multilocularis. This could be a source of larval infection
to P. maniculatus and other insectivorous rodents. This is another
source of infection that could increase risk of human exposure. The
ever-increasing growth of cities also increases the risk of human
contact with fecal contamination originating from sylvatic and
domestic definitive hosts.

Diagnosis of alveolar hydatid disease is difficult even in regions
where clinicians are experienced (31). However, Rausch (33) summarized
available information on human infection with E. multilocularis and
listed 18 confirmed cases in Eskimos in Alaska. At times even the
postmortem diagnosis of alveolar hydatid disease is difficult, and
pathologists unfamiliar with it are liable to make incorrect diagnoses,
this being especially true in the continental United States where the disease is rarely observed (31).

The early stages of E. multilocularis larval infection in humans have not been studied. However in experimental infections, after penetrating the intestinal wall, the oncospheres make their way to the liver by means of the portal circulation (31). The larvae proliferate by exogenous budding, but the rate of growth in humans appears to be much slower than in natural hosts (loc. cit.).

The liver is the organ most commonly affected in humans, the degree of pathogenicity being related to the location of the cyst within the liver (loc. cit.). E. multilocularis infection gives rise to a chronic, afebrile disease characterized by hepatomegaly, with icterus and ascites in the terminal stages (loc. cit.). Alveolar hydatid disease is sometimes termed malignant because the larval growth resembles a neoplasm, and infections are difficult to distinguish from carcinoma of the liver. Radical surgery of the affected areas is the usual treatment. However, most cases are far advanced when diagnosed and the prognosis is unfavorable; the majority ending fatally (loc. cit.).

Preventive measures for alveolar hydatid disease are complicated by the sylvatic nature of the cycle. Lukashenko (26) stated that the preventive measures consist mainly of the interruption of food contact between dogs and cats and the source of their rodents infection.
Rausch (31) stressed the importance of fecal contamination and of hygiene in handling of carnivore furs. The importance of developing an anthelmintic effective against *E. multilocularis* can not be over stressed. However, to date a satisfactory drug has not been available. The difficulty in developing a useful anthelmintic is related to the tendency of *Echinococcus multilocularis* to embed in the mucosa of the intestine, so that direct action of the drug is hampered (26). People living in areas where *E. multilocularis* is endemic should be informed about this disease and the use of proper hygiene, and restrictions of house pet activities should be stressed.
In 1976 a survey was initiated to determine the intermediate host(s) of the cestode *Echinococcus multilocularis* in southwestern Montana. Five species of intermediate hosts were examined. Two muskrats with *E. multilocularis* liver cysts were found in Gallatin and Madison counties in December, 1977. This is the first confirmed natural infection with this larval cestode in the muskrat to be reported in North America. It is my hypothesis that during the 1977 periods of reduced streamflow, muskrats in southwestern Montana were stressed, forced onto the land and subjected to heavy predation by foxes. The muskrats became infected by accidently ingesting ova while foraging. Evidence to support this hypothesis include:

1. The natural barriers between muskrats and canine or feline predators were reduced during 1977 as muskrats were depending more upon terrestrial resources.

2. Although natural infections have not been reported in muskrats in North American, experimental infections in muskrats (30) have been successful.

3. The habitats surveyed in this project may support populations of a wide variety of rodent intermediate hosts, including deer mice.
The recovery of 2 muskrats naturally infected with larval E. multilocularis does not indicate this rodent is the principle intermediate host, but it suggests that under certain environmental conditions muskrats may become an important link in the chain of transmission.

The successful infection of three kittens with metacestodes obtained from muskrat liver cysts indicated:

1. The protoscolices were viable and could be identified biologically as Echinococcus multilocularis.
2. The isolate of this cestode in southwestern Montana is infective for the domestic cat.

Possible implications for human exposure to E. multilocularis in southwestern Montana are discussed.
APPENDIX

The survey to determine potential intermediate host(s) of the cestode *Echinococcus multilocularis* involved identification of other parasites present in the livers of mammals examined. In this appendix, three metazoan parasites recovered from Montana rodents will be discussed: the nematode *Capillaria hepatica*; the larval cestode *Taenia taeniaeformis*; and an unidentified cestode having a coenurus type larva.

*Capillaria hepatica* is a nematode that occurs as an adult in the liver of numerous species of mammals. This nematode has been reported in a number of mammalian species including humans. However, it is primarily a parasite of rodents (37). *Capillaria hepatica* is cosmopolitan in distribution (9). The life cycle (Fig. 8) suggests that predation is the main mode of transmission (37). In 1960, Freeman and Wright proposed the life cycle in Fig. 9, because their data showed predation could not account for the patterns of infection observed in rodents in Algonquin Park, Canada (10). Data collected by Farhang-Azad (9) correlated closely with the hypothesis of Freeman and Wright.

Data on the prevalence of *Capillaria hepatica* in muskrats are presented in Table 3. Data on the prevalence of this nematode in other mammals collected are in Table 2. The map in Fig. 10 shows
1) eaten by carnivore or
2) released through decomposition

Fig. 8. The transmission of *Capillaria hepatica* is through predation. (From Animal Parasites: Their Biology and Life Cycles. W. Olsen, 1962, p. 324.)
Primary Source of Infection

1. cannibalism
2. unembryonated ova passed in feces of rodent
3. coprophagy after embryonation occurs

Secondary Source of Infection

1. predation of carnivore on infected rodent
2. unembryonated ova passed in feces of carnivore
3. coprophagy after embryonation occurs

Fig. 9. Life cycle of Capillaria hepatica as proposed by Freeman and Wright. (J. of Parasitol. 46: 373-382. 1960).
Fig. 10. Collecting sites where small mammals positive for _C. hepatica_ infections were trapped.
Table 2. Prevalence of *Capillaria hepatica* in mammals from Gallatin County, Montana.

<table>
<thead>
<tr>
<th>Animal Examined</th>
<th># Infected with <em>C. hepatica</em></th>
<th>Total # examined</th>
<th>% infected with <em>C. hepatica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>House Mouse</td>
<td>2</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>(<em>Mus musculus</em>)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vole</td>
<td>0</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>(<em>Microtus spp.</em>)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deer mouse</td>
<td>16</td>
<td>118</td>
<td>14</td>
</tr>
<tr>
<td>(<em>Peromyscus maniculatus</em>)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shrew</td>
<td>0</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>(<em>Sorex sp.</em>)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Prevalence of three metazoan liver parasites of muskrats in relation to collecting areas.

<table>
<thead>
<tr>
<th></th>
<th>Coenurus Larvae</th>
<th>Hydatigera (=Taenia)</th>
<th>Capillaria hepatica</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1976</td>
<td>1977</td>
<td>Total</td>
</tr>
<tr>
<td>East Gallatin</td>
<td>12/28 3/16 15/44 34</td>
<td>0/28 1/16 1/44 2</td>
<td>0/28 4/16 4/44 9</td>
</tr>
<tr>
<td>North of Bozeman</td>
<td>0 2/6 2/6 33</td>
<td>0 1/6 1/6 17</td>
<td>0 0/6 0/6 0</td>
</tr>
<tr>
<td>Missouri</td>
<td>7/33 2/7 9/40 23</td>
<td>2/33 1/7 3/40 8</td>
<td>0/33 1/7 1/40 3</td>
</tr>
<tr>
<td>North of Belgrade</td>
<td>0 0/1 0/1 0</td>
<td>0 0/1 0/1 0</td>
<td>0 0/1 0/1 0</td>
</tr>
<tr>
<td>Bozeman</td>
<td>0 2/5 2/5 40</td>
<td>0 1/5 1/5 20</td>
<td>0 0/5 0/5 0</td>
</tr>
<tr>
<td>Madison</td>
<td>10/45 2/13 12/58 21</td>
<td>0/45 0/13 0/28 0</td>
<td>0/45 0/13 0/58 0</td>
</tr>
<tr>
<td>Central Park</td>
<td>1/12 0/2 1/15 7</td>
<td>1/12 0/2 1/15 7</td>
<td>0/13 0/2 0/15 0</td>
</tr>
<tr>
<td>Upper Gallatin</td>
<td>0 0/1 0/1 0</td>
<td>0 0/1 0/1 0</td>
<td>0 0/1 0/1 0</td>
</tr>
<tr>
<td>South of Belgrade</td>
<td>0/10 0/10 0</td>
<td>0/10 0/10 0</td>
<td>2/10 0 2/10 20</td>
</tr>
<tr>
<td>Upper Madison</td>
<td>1/12 5/12 42</td>
<td>0/12 0 0/12 0</td>
<td>0/12 0 0/12 0</td>
</tr>
<tr>
<td>Total</td>
<td>44/192 23</td>
<td>7/192 4</td>
<td>7/192</td>
</tr>
</tbody>
</table>
the focal distribution of this parasite in Gallatin County. The data presented here regarding the epizootiology of Capillaria hepatica, correlates with the life cycle in Fig. 9.

Farhand-Azad noted the abundance of rats in crowded urban areas to be of considerable importance in the transmission of C. hepatica, to children (9). It is important to note the infection of one house mouse with this parasite in Montana. In Gallatin county, human exposure to C. hepatica is possible through fecal contamination of soil and household areas from release of ova in feces from dogs, cats, and house mice. No evidence of human infection acquired in Montana has been reported in the literature.

The cestode larva of Hydatigera (=Taenia) taeniaeformis was observed in 4 of 18 voles collected 2 miles southwest of Bozeman in an irrigated hayfield. In a survey in southwestern Montana (27), the prevalence of infection of voles with H. taeniaeformis varied with the proximity of voles to large numbers of primary hosts such as the domestic cat (27). Data collected in this survey support his findings. Prevalence of this strobilocercus larva in muskrats is shown in Table 3. The larval H. taeniaeformis is a bladderworm that develops in the liver of various rodent intermediate hosts (28). The strobilocercus must be ingested through predation or scavenging by the final host for the adult cestode to develop.
Coenurus larval cestodes were recovered in 23% of the muskrats. Ameel (2) described a proliferating cestode larvae located in 'cysts' formed by walls of host tissue, with more than one scolex present in each bladder. His data include pictures of whole cysts which are similar to the cysts recovered in this survey. The photomicrograph in Fig. 12 is a cross section of a coenurus cyst dissected from host tissue. Ameel (loc. cit.) was unable to infect a cat with cyst material and suggested the possibility of a raptorial bird as the final host. Dr. W. L. Jellison (14) suggested the mink as a possible final host for this cestode.

The data presented in this appendix on three liver metazoan parasites are obviously incomplete. It is hoped that additional interest in research on the ecology and transmission of these parasites and their hosts will be stimulated.
Fig. 11. *C. hepatica* ova in naturally infected muskrat liver; approximately X200.

Fig. 12. Cross section of a coenurus cyst dissected from host tissue; approximately X12.
LITERATURE CITED


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  Studies on intermediate hosts of Echinococcus multilocularis...