Studies on native small mammals as intermediate hosts of Echinococcus multilocularis
by Harvey Peter Feigley

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Zoology
Montana State University

Abstract:
A field survey to determine the intermediate host(s) of Echinococcus multilocularis in southwestern Montana involved the examination of 1,245 small mammals representing 17 species. Two naturally infected muskrats (Ondatra zibethicus) were collected from the East Gallatin River in Gallatin County during the winter of 1980. No other species were found to be infected with this larval cestode. Fifty-eight experimental inoculations of 5 species of rodents and 1` lagomorph by feeding ova induced fertile cyst development in one deer mouse (Peromyscus maniculatus) and one muskrat. Successful culturing of the Montana isolate by intra-peritoneal inoculation of cyst material occurred in 4 of 11 cotton rats (Sigmodon hispidus). Nineteen deer mice were refractory to infection via intraperitoneal injection. Additionally, data on the occurrence of 3 metazoan liver parasites in Montana small mammals is included.

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STUDIES ON NATIVE SMALL MAMMALS AS INTERMEDIATE HOSTS OF *ECHINOCOCCUS MULTILOCULARIS*

by

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

of

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in

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ABSTRACT

A field survey to determine the intermediate host(s) of Echinococcus multilocularis in southwestern Montana involved the examination of 1,245 small mammals representing 17 species. Two naturally infected muskrats (Ondatra zibethicus) were collected from the East Gallatin River in Gallatin County during the winter of 1980. No other species were found to be infected with this larval cestode. Fifty-eight experimental inoculations of 5 species of rodents and 1 lagomorph by feeding ova induced fertile cyst development in one deer mouse (Peromyscus maniculatus) and one muskrat. Successful culturing of the Montana isolate by intraperitoneal inoculation of cyst material occurred in 4 of 11 cotton rats (Sigmodon hispidus). Nineteen deer mice were refractory to infection via intraperitoneal injection. Additionally, data on the occurrence of 3 metazoan liver parasites in Montana small mammals is included.

INTRODUCTION

The life cycle of *Echinococcus multilocularis* Leuckart, 1863 (Cestoda: Taeniidae) has only recently been described and current studies of distribution and dynamics of infection are extending the knowledge of the species. The adults of this tapeworm are found in the small intestine of foxes and related carnivores. Eggs passed with the feces are then ingested by a suitable intermediate host, primarily rodents. The eggs hatch in the small intestine, releasing hexacanth larvae which penetrate the intestinal wall and enter the portal circulation. The larvae most often invade the liver where development of a multilocular (alveolar) type hydatid cyst occurs. Larval development may occur in the liver or other organs of humans who accidentally ingest *E. multilocularis* ova, resulting in a condition known as alveolar hydatid disease.

First recognition of the etiological agent of alveolar hydatid disease in man was by Virchow, in 1855. He reported cases of apparent liver malignancy caused by the growth of a larval cestode of the genus *Echinococcus* (Rausch, 1956). Owing to the characteristic multilocular appearance of the larval cysts, the name *Echinococcus multilocularis* was designated by Leuckart in 1863. The relationship of this organism to its adult form was a matter of controversy for a number of years because neither the adult nor the life cycle had been described. At that time, alveolar hydatid disease was believed

to be restricted to Eurasia. The fact that Echinococcus granulosus was the only species recognized in Europe at that time added to the confusion. However, the larval cyst of E. granulosus is a unilocular fluid-filled cyst which differs significantly from the multilocular cyst of E. multilocularis.

In 1951, Rausch and Schiller mistakenly reported E. granulosus cysts from a tundra vole (Microtus oeconomus) on St. Lawrence Island in the Bering Sea. Ensuing experimental studies of the larval development of the species of Echinococcus found on St. Lawrence Island and comparison with the development of the larva of E. granulosus demonstrated substantial differences between the two. Among these differences were that the larva of Echinococcus species on St. Lawrence Island occurred naturally in rodents rather than ungulates, and that the alveolar type of larva was produced by exogenous budding (Rausch, 1954). The dissimilarities between the two larval forms led to the description of a new species, Echinococcus sibiricensis, by Rausch and Schiller (1954). They suggested that E. granulosus reported in muskrats from Russia may in fact be E. sibiricensis. They also recognized that the larval cestode found on St. Lawrence Island closely resembled the alveolar larva of Echinococcus occurring in man in Eurasia and suggested that the two may be identical.

Through an investigation of alveolar hydatid disease in Germany, Vogel, in 1955, identified a morphological and ecological equivalent

of E. sibiricensis and pointed out that the name Echinococcus multilocularis, applied by Leuckart in 1863, was the valid designation based on priority (Rausch, 1958). Rausch (1956) then confirmed Vogel's findings. With the identity of E. multilocularis finally settled further studies were initiated to reveal the distribution of E. multilocularis in North America and evaluate the potential for human exposure.

In 1956, Rausch found the distribution of E. multilocularis to include the mainland of Alaska and at that time alluded to the fact that favorable conditions for the establishment of the life cycle existed in Canada and the contiguous United States. Fay and Williamson (1962) found E. multilocularis to be abundant on the Pribilof Islands in the Bering Sea. Choquette et al. (1962) reported E. multilocularis from Eskimo Point, on the western coast of Hudson Bay, Northwest Territories. This was the first record of the cestode occurring on the Canadian mainland. Further examination in Canada showed the life cycle to be established in Manitoba (Lee, 1969; Leiby et al., 1969; Baron, 1970), Saskatchewan (Hnatiuk, 1966; 1969; Wobesser, 1971), and Alberta (Holmes et al., 1971; Chalmers et al., 1974). E. multilocularis was first found in the contiguous United States by Leiby and Olsen (1964) in North Dakota. The known distribution in the United States was then expanded to include Minnesota (Carney and Leiby, 1968; Leiby et al., 1970), South Dakota

(Leiby et al., 1970), Iowa (Leiby et al., 1970), Montana (Leiby et al., 1970; Seesee and Worley, 1976; Eastman and Worley, 1979), and Wyoming (Kritsky et al., 1977).

The adult infection seems to be fairly specific for carnivores of the family Canidae, but occasional infections of domestic felids have been reported. Both the arctic fox (Alopex lagopus) and the red fox (Vulpes vulpes) have been shown to be important definitive hosts for E. multilocularis in Alaska (Rausch, 1967) and Canada (Choquette et al., 1962; Hnatiuk, 1969). Where E. multilocularis exists in the contiguous United States, the red fox serves as the principal definitive host along with the coyote (Canis latrans) (Leiby et al., 1970; Seesee et al., 1979). Domestic dogs (Canis familiaris) infected with E. multilocularis have been reported from Alaska (Rausch, 1956; Fay, 1973). Naturally infected domestic cats (Felis catus) have been reported from Canada (Wobesser, 1971) and the United States (Leiby and Kritsky, 1972). The larval stage of E. multilocularis has been found naturally to infect a wide variety of rodent hosts, with microtine and cricetine rodents being the most commonly reported (Rausch and Schiller, 1951; Thomas et al., 1954; Fay and Williamson, 1962; Hnatiuk, 1966; Leiby et al., 1970; Eastman and Worley, 1979; Kritsky et al., 1977). Insectivores of the genus Sorex also have been found to serve as intermediate hosts in Alaska (Thomas et al., 1954; Fay and Williamson, 1962).

Humans occasionally become accidental intermediate hosts for E. multilocularis. Human infection most likely occurs when contaminated water, fruits, or vegetables are consumed. The handling of fox and coyote carcasses and furs is also a likely means of human contact with tapeworm ova. The fact that domestic dogs and cats may harbor the adults of E. multilocularis greatly increases the chance of human exposure to viable ova. Eskimos in Alaska have been shown to be a high risk group of people since they have a close association with sled dogs which feed freely on voles (Fay, 1973). Cases of human alveolar hydatid disease have been reported from Canada and the United States, but there is suspicion that most of these cases were acquired outside of North America (Polley, 1978). In 1977, a case of alveolar hydatid disease was confirmed in a woman from Minnesota. The source of infection was assumed to be a pet dog or cat. This constitutes the first record of an autochthonous case of alveolar hydatid disease in the contiguous United States (Gamble et al., 1979). Diagnosis of alveolar hydatid disease is often difficult since the disease closely resembles carcinoma of the liver (Rausch, 1958). Due to the difficulty of diagnosis, it is probable that locally acquired cases of alveolar hydatid disease may have been overlooked. The potential for establishment of a domestic life cycle poses a health problem due to the zoonotic transmission of E. multilocularis.

Following the first report of naturally infected deer mice from eastern Montana (Leiby et al., 1970), studies were initiated to determine further the extent of the distribution of the cestode in Montana. Seesee and Worley (1976) reported the occurrence of E. multilocularis in red foxes from southwestern Montana. A survey of Montana coyotes by Seesee et al. (1979) showed coyotes to be infected with the cestode. During a study to determine the intermediate hosts of E. multilocularis in Montana, Eastman and Worley (1979) reported the first North American record of muskrats harboring the larval infection. To date, E. multilocularis appears to be restricted to the portion of Montana east of the continental divide (Sterner et al., unpublished data).

The purpose of this study was to reveal the extent of involvement of native small mammals in the maintenance of the life cycle of E. multilocularis. Experimental infections were also attempted in an effort to culture the Montana isolate of E. multilocularis and test the susceptibility of two native rodents and several exotic small mammal species to E. multilocularis infection.

MATERIALS AND METHODS

Native rodents and insectivores were trapped with box traps and Victor snap traps during late spring, summer, and early fall of 1980. Mammals which were alive were killed in the field by cervical dislocation. Trapping was conducted in areas of Gallatin and Fergus counties, primarily where infected red foxes had previously been collected. The sites trapped in Gallatin Co. included riparian forest, riparian shrubland, coniferous forest, and irrigated farmland, while the sites in Fergus Co. consisted of riparian shrubland and dryland wheat farmland. Richardson's ground squirrels (Spermophilus richardsoni) from Gallatin Co. and whitetail jackrabbits (Lepus townsendii) from Fergus Co. were collected with a .22 caliber rifle and 12 gauge shotgun respectively. Muskrat (Ondatra zibethicus) and beaver (Castor canadensis) carcasses were donated by local trappers during November and December of 1979 and 1980 and January, February, and March of 1980 and 1981. These carcasses were either refrigerated or frozen until necropsy. Muskrats and beavers were collected from Gallatin, Madison, Jefferson, Broadwater, Park, Fergus, and Phillips Counties.

At necropsy, all mammals were examined internally for larvae of E. multilocularis. Liver, heart, lungs, spleen, kidneys, body cavity, and associated mesenteries and connective tissue were examined macroscopically for evidence of E. multilocularis. All tissues with

suspected lesions were excised and fixed in 10% buffered formalin. Rausch and Schiller (1956) determined that lesions in the livers of voles experimentally infected with eggs could be detected macroscopically at 48 hrs postinfection. In cases where obvious cysts of E. multilocularis were found, the tissue was placed in a 0.86% saline solution and used for experimental purposes. A portion of the cyst and adjacent host tissue was also fixed in 10% buffered formalin. Other liver parasites encountered were identified and recorded (Appendix, Table III). Formalin fixed tissues containing E. multilocularis and other parasites were embedded in paraplast, sectioned at 5 μm, and stained with hematoxylin and eosin. The necropsy records kept for each mammal specimen included a identification number, location where the mammal was collected, sex, and parasite data. The species of small mammals examined were determined by referring to Hoffman and Pattie (1968).

From December, 1979 to July 1981, 91 experimental infections were attempted in an effort to culture the Montana isolate of E. multilocularis and to test the susceptibility of various rodents and lagomorphs to infection. Three methods of infection were employed: 1) the feeding of gravid proglottids to rodents and lagomorphs; 2) intraperitoneal injection of cyst material from naturally and experimentally infected rodents; 3) surgical implantation of a small intact cyst into the peritoneal cavity of rodents. Table II lists the

species, number of experimental cases, the method of infection, and dosages for the 91 experimental animals. Of 31 deer mice (Peromyscus maniculatus) fed gravid proglottids, 10 were fed 1 gravid proglottid, while the other 21 deer mice along with 6 cotton rats (Sigmodon hispidus), 8 gerbils (Meriones unguiculatus), and 4 hamsters (Mesocricetus auratus) were all fed 3 gravid proglottids each. Five European rabbits (Oryctolagus cuniculus) and 4 muskrats were fed 5 gravid proglottids each. All animals, except the muskrats, received their inoculum via a stomach tube and a one ml syringe. In order to inoculate the muskrats, they were deprived food for one day and then each was fed a piece of carrot containing 5 proglottids. This resulted in the immediate consumption of the carrots containing proglottids.

Intraperitoneal injection of cyst material has been shown to be an effective means of lateral transmission of the larval stage of E. multilocularis (Norman and Kagan, 1961; Kagan et al., 1965; Hinz, 1972). Cyst material from 2 naturally infected muskrats was injected intraperitoneally into 15 deer mice. The dosages contained 8,700 to 17,500 protoscolices per animal. Cyst material from an experimentally infected deer mouse was injected intraperitoneally into 4 deer mice and 3 cotton rats, with each animal receiving approximately 900 protoscolices. The remaining 11 cotton rats were inoculated with cyst material from an experimentally infected muskrat.

Three of these cotton rats received a small intact cyst which was surgically implanted into the peritoneal cavity. The other 8 cotton rats were injected intraperitoneally with 0.5 gm of diced cyst material in one ml of .86% saline solution, following a procedure described by A. Marchiondo (1981). The viability of inocula containing protoscolices was confirmed by the observed movement of the protoscolices.

Domestic cats have been shown to serve as suitable definitive hosts for E. multilocularis both naturally (Wobesser, 1971; Leiby and Kritsky, 1972) and experimentally (Rausch and Richards, 1971; Eastman and Worley, 1979). Based on this information, 2 domestic cats were fed protoscolices to confirm the susceptibility of cats to the Montana isolate of E. multilocularis. One cat received 2 doses of protoscolices from a naturally infected muskrat on 2 consecutive days. The doses consisted of 28,500 and 17,200 protoscolices respectively. Both doses were mixed with canned cat food. The second cat received one dose of 900 protoscolices from an experimentally infected deer mouse via a stomach tube and a 35 ml syringe. The inocula given to both cats contained viable protoscolices. Both cats were killed at 28 days postinfection. Rausch and Schiller (1956) found the patent period of E. multilocularis in experimentally infected arctic foxes to be 32 to 33 days. The small and large intestines were removed from the cats, cut open

longitudinally, and the contents scraped into buckets. The intestinal contents were then washed through a 40 mesh screen to remove large debris and then backwashed through a 100 mesh screen. The contents retained by the 100 mesh screen were washed into one quart jars, poured into petri dishes, and examined under a binocular microscope for adult E. multilocularis.

RESULTS

Field Survey

The results of the collection and necropsy of potential intermediate hosts of E. multilocularis are summarized in Table I. A total of 1,245 small mammals representing 3 orders and 17 species was examined from 7 Montana counties. The larva of E. multilocularis occurred in 2 muskrats which were collected from the same location on the East Gallatin River, approximately 15 km northwest of Bozeman, Montana. All other small mammals including 189 deer mice, voles, and shrews which were trapped near the location where the naturally infected muskrats were collected showed no evidence of E. multilocularis infection.

Figure 1 is a photomacrograph of the liver of one of the naturally infected muskrats from the East Gallatin River. Two separate foci of cyst development were evident and measured 28x31 mm and 25x30 mm on the hepatic surface. Numerous daughter cysts could be seen which gave the cyst the characteristic multilocular appearance. The other naturally infected muskrat had only one focus of infection in the liver measuring 29x47 mm. In both cases the cysts contained viable protoscolices.

Experimental Infections

Table II summarizes the experimental results. One deer mouse and one muskrat were successfully infected with larval E. multilocularis

TABLE I. Prevalence of Larval *E. multilocularis* in 1,245 Montana Small Mammals.

Taxonomic Classification	No. Infected / No. Examined
Order Insectivora	
Soricidae	
Sorex vagrans	0/102
Sorex cinereus	0/2
Sorex palustris	0/6
Order Rodentia	
Cricetidae	
Cricetinae	
Peromyscus maniculatus	0/208
Microtinae	
Ondatra zibethicus	2/647
Microtus longicaudus	0/21
Microtus montanus	0/1
Microtus pennsylvanicus	0/95
Clethrionomys gapperi	0/23
Arvicola richardsoni	0/2
Muridae	
Mus musculus	0/9
Castoridae	
Castor canadensis	0/77
Zapodidae	
Zapus princeps	0/16
Sciuridae	
Spermophilus richardsoni	0/20
Eutamias amoenus	0/8
Geomyidae	
Thomomys talpoides	0/1
Order Lagomorpha	
Leporidae	
Lepus townsendii	0/7
	2/1245(0.16%)

TABLE II. Experimental Inoculations of Rodents and Lagomorphs with E. multilocularis Eggs or Cyst Material.

Species	Route of inoculation	Positive Cases No. inoculated	Dosage
Deer Mouse (Peromyscus maniculatus)	oral	1/31	1-3 proglottids
	*I.P.	0/15	8,700-17,500 protoscolices
	I.P.	0/4	900 protoscolices
Gerbil (Meriones unguiculatus)	oral	0/8	3 proglottids
Hamster (Mesocricetus auratus)	oral	0/4	3 proglottids
Muskrat (Ondatra zibethicus)	oral	1/4	5 proglottids
Cotton Rat (Sigmodon hispidus)	oral	0/6	3 proglottids
	I.P.	+/3	900 protoscolices
	I.P.	2/8	0.05 gm cyst material
	**S.I.	2/3	1 intact cyst
European Rabbit (Oryctolagus cuniculus)	oral	0/5	5 proglottids

*I.P. = Intraperitoneal injection
**S.I. = Surgical implantation
+ Cases still incubating

by the feeding of gravid proglottids. The photomacrograph in Fig. 2 shows the liver of an experimentally infected deer mouse which was inoculated with 3 gravid proglottids and allowed to incubate for 150 days. Two foci of infection were present, measuring 6x8 mm and 7x8 mm. Both cysts projected well above the hepatic surface and although they lacked multilocular structure, viable protoscolices were present. A section through a portion of a cyst from the deer mouse liver is seen in Fig. 3. Three layers of tissue were clearly discernible. The outermost layer of tissue consisted of hepatic parenchyma. The next layer was composed of connective tissue surrounding the developing cyst. Within this fibrotic tissue were many polymorphonuclear granulocytes, primarily eosinophils, which had migrated to the area in response to the parasitic infection. According to Ohbayashi et al. (1971), eosinophils constitute the majority of inflammatory cells within the connective tissue capsule surrounding the cyst. The third and innermost layer constituted the germinal membrane. Many active sites on the germinal membrane were visible, and it was at these active sites that protoscolices were formed. Protoscolices were present within the cyst cavity, and hooks were visible on a few of them.

The experimentally infected muskrat, after receiving 5 gravid proglottids, was held 90 days prior to necropsy. Figure 4 shows the extent of infection in the liver of the experimentally infected

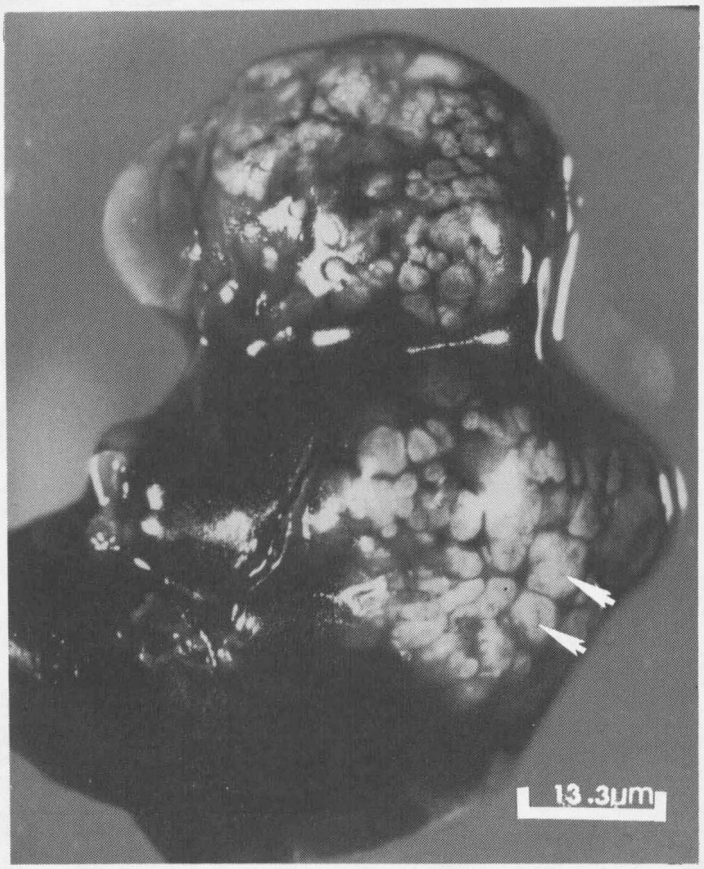

Figure 1. Photomacrograph of a muskrat liver naturally infected with larval E. multilocularis. Arrows (↗) point to daughter cysts.

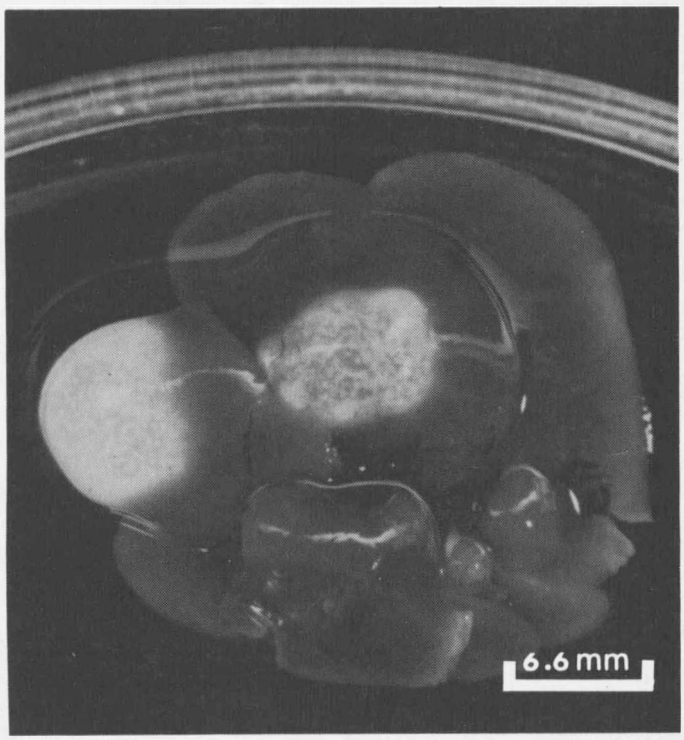

Figure 2. Photomacrograph of a deer mouse liver experimentally infected with larval E. multilocularis.

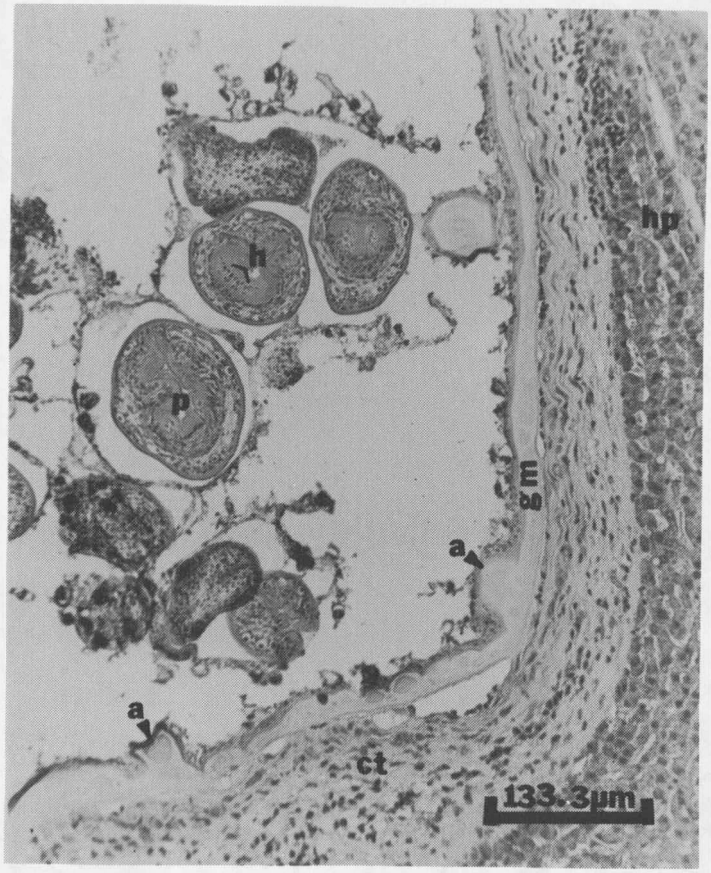

Figure 3. Section through an E. multilocularis cyst from an experimentally infected deer mouse. hp = hepatic parenchyma; ct = connective tissue; a = active sites; gm = germinal membrane; p = protoscolex; h = hooks.

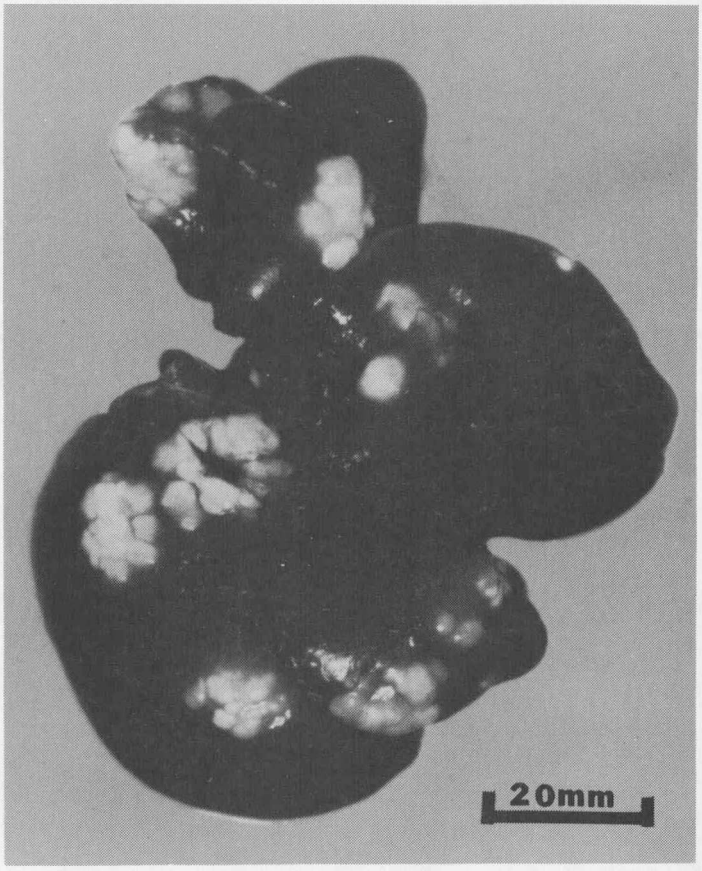

Figure 4. Photomacrograph of a muskrat liver experimentally infected with larval E. multilocularis.

muskrat. Numerous foci of infection were present, with all lobes of the liver being involved. The size and structure of the cysts varied from a 3x5 mm unilocular cyst to a 15x18 mm multilocular cyst. Protoscolices from the developing cysts were viable. Since the experimental muskrats were trapped from a natural population there was no assurance that they had not been previously exposed to E. multilocularis ova. However, over 200 muskrats were examined from the area where the 4 experimental muskrats were collected and showed no signs of infection. Therefore, the chance of previous exposure of the experimental muskrats was minimal.

A section through a portion of a liver cyst from the experimentally infected muskrat is shown in Fig. 5. The same 3 tissue layers were present as described in the deer mouse liver cyst. However, the larval growth in the muskrat was much more extensive and several loculi were present, each with its own germinal membrane. The loculi, which were the product of exogenous budding, contained numerous protoscolices. During necropsy of the experimentally infected muskrat secondary metastatic cysts were found embedded in adipose tissue surrounding the right kidney and in mesenteric tissue near the genitalia. The two cysts near the kidney were small, unilocular, and apparently sterile. The cyst from the mesenteric tissue (Fig. 6) was multilocular, contained a few developing protoscolices, and the germinal membrane was quite thin.

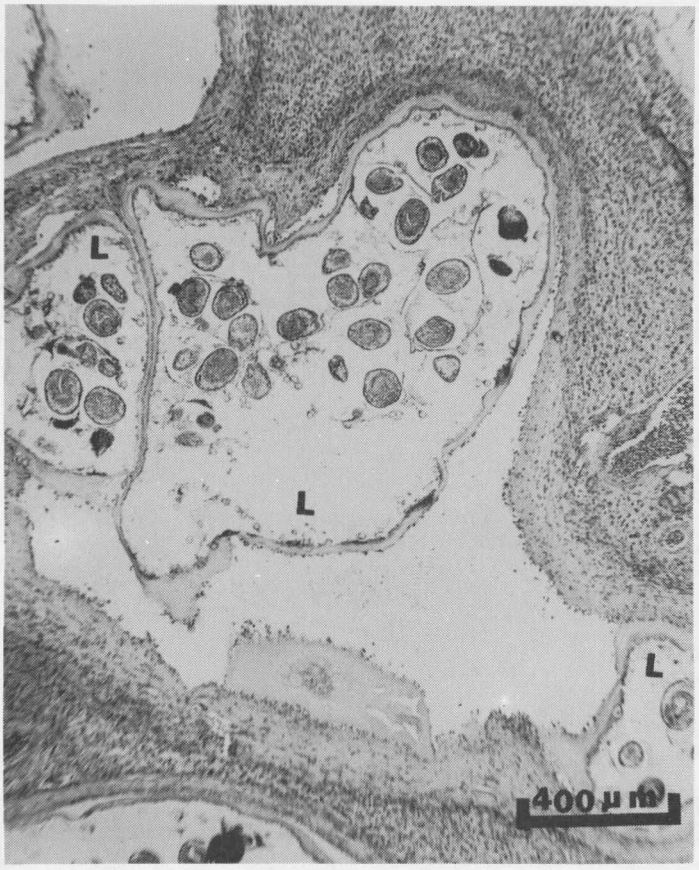

Figure 5. Section through an E. multilocularis cyst with several loculi (L), from the liver of an experimentally infected muskrat.

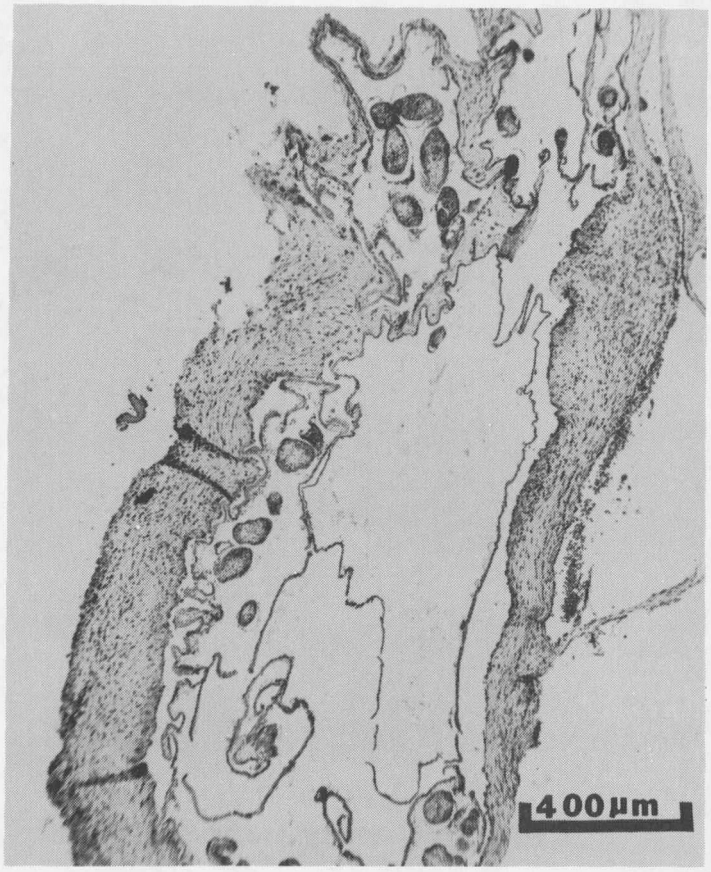

Figure 6. Section through an E. multilocularis cyst located in the mesenteric tissue near the genitalia of an experimentally infected muskrat.

Attempts to produce larval infections in gerbils, hamsters, cotton rats, and European rabbits with E. multilocularis ova were unsuccessful. Also, intraperitoneal injection of saline suspensions of diced cyst into 19 deer mice failed to induce larval infections. Viable cysts developed in 4 of 11 cotton rats that were inoculated intraperitoneally. Three inoculated cotton rats were left to incubate for a longer period of time. The Montana isolate of E. multilocularis will be maintained at Montana State University by Dr. D. E. Worley and co-workers.

Efforts to infect 2 adult domestic cats with E. multilocularis by oral inoculation of viable protoscolices were unsuccessful.

DISCUSSION

The life cycle of *E. multilocularis* involves a predator-prey relationship, and the prevalence of infection in the intermediate host is influenced by the type of predator-prey relationship, along with the habitat, and the numerical densities of both the intermediate and final hosts. Due to the influence of these variables, the life cycle of *E. multilocularis* may differ slightly with regard to the intermediate host from one ecosystem to another.

Both the field and experimental results obtained during this study indicate that the life cycle of *E. multilocularis* in southwestern Montana differs with respect to the intermediate hosts when compared with other enzootic areas. In Alaska, where *E. multilocularis* is quite prevalent, voles are the principal intermediate host, and although other species of small mammals are found to be naturally infected, their importance in maintaining the life cycle is minimal (Rausch, 1967). In north central United States and southern Canada, investigations of the cestode indicate that the deer mouse is the principal natural intermediate host of *E. multilocularis* (Leiby et al., 1969; 1970; Rausch and Richards, 1971). Leiby and Nickel (1968) suggested that populations of deer mice are more stable than vole populations and therefore play a more important role in maintaining the life cycle of *E. multilocularis* in North Dakota. In eastern Montana, the deer mouse has been shown to

be naturally infected with E. multilocularis (Leiby et al., 1970). The results of the present study along with the results of a less extensive field study by Eastman and Worley (1979) indicate that the muskrat is involved in the life cycle of E. multilocularis in southwestern Montana. Neither study found any other species serving as intermediate hosts.

The susceptibility of the muskrat to infection has been demonstrated experimentally by Ohbayashi et al. (1971) and by the results of this study. The muskrat has been found to be an important intermediate host of E. multilocularis in some isolated mountain valleys of Russia (Shpil'ko and Koval'chuk, 1973). Thus far, southwestern Montana is the only area in the North American range of E. multilocularis where naturally infected muskrats have been reported.

In areas of southwestern Montana where suitable habitats support large muskrat populations, the fox-muskrat pathway of the life cycle of E. multilocularis may be an important one. The Gallatin and Madison River valleys, where infected muskrats have been reported, consist of river bottom, marshes, ponds, and spring creeks which are ideal for supporting large muskrat populations. Foxes are opportunistic predators and tend to prey upon food sources which are readily available (Scott, 1955). Foxes have also been shown to be important predators of muskrats (Heit, 1944; Errington, 1945). This evidence along with the fact that no other infected small mammals

were trapped from the area where the 2 infected muskrats were collected indicate the importance of the fox-muskrat cycle in southwestern Montana.

Ables (1969) demonstrated that the home ranges of foxes inhabiting areas of great ecological diversity were smaller than home ranges of foxes in less diverse areas. Southwestern Montana consists of diverse habitat types including coniferous forest, riparian forest, riparian shrubland, sagebrush grassland, and agricultural land as opposed to the relatively large homogenous regions of eastern Montana and North Dakota (Küchler, 1974). Because of the diversity of habitat types in southwestern Montana, foxes inhabiting an area which supports a large muskrat population are less likely to travel large distances and thereby locally maintain the fox-muskrat life cycle.

The different habitat types of southwestern Montana support a wide varity of small mammal species (Hoffmann and Pattie, 1968). The diverse mammalian fauna may facilitate the existence of alternate pathways in the life cycles of E. multilocularis. Also, in North Dakota, Leiby and Kritsky (1974) found the prevalence of infection in the intermediate host varied by habitat. Therefore, it is possible that different habitat types of southwestern Montana where E. multilocularis is present, may exhibit different prevalences of infection.

During this study, the apparent prevalence of larval E. multilocularis was found to be 0.16%. Typically, in areas where E. multilocularis is established, a much higher percentage of the local small mammal population is infected with the larval cestode. On St. Lawrence Island, Rausch and Schiller (1956) found 2% of the voles examined to be infected with larval E. multilocularis when 40% of foxes harbored the adult. Leiby (1966) reported 4.7% of 832 rodents examined from North Dakota to be infected with the larval stage.

Several explanations exist to account for the failure to trap infected insectivores and rodents other than muskrats. Leiby and Kritsky (1974) found that the highest prevalence of the larval cestode in deer mice occurred in early spring. During that time, the deer mouse population consisted primarily of overwintering adults having a greater probability of being infected. Since the field trapping of small mammals in the current study occurred in late spring, summer, and early fall, it is likely that dilution of the sample with juveniles decreased the probability of trapping infected rodents and insectivores. Also, the prevalences of in-infection in foxes both state-wide and in southwestern Montana are 17.3% and 22% respectively (Sterner et al., unpublished data). Due to the relatively low prevalence of infection in foxes, one

would expect the occurrence of the larval cestode also to be low. It is likely that in order to find rodents, other than muskrats, and insectivores infected with larval E. multilocularis, a much larger sample of the small mammal population may be necessary.

The fact that muskrats were the only intermediate host recorded in southwestern Montana may indicate that there is a difference between the Montana strain of E. multilocularis and the strains found in North Dako-a and Alaska. Experimentally, the muskrat was found to be quite susceptible to the Montana strain of E. multilocularis, based upon limited observations involving only 4 animals. The pathogenicity and rate of cyst development were much greater in the muskrat than in the deer mouse. While comparing the Alaska and North Dakota strains of E. multilocularis, Rausch and Richards (1971) found indications of strain differences with respect to infectivity in various rodent species. Their results showed deer mice to be more susceptible to the North Dakota strain than the Alaska strain, which supported the idea that the deer mouse was the principal intermediate host in North Dakota. Results from the current study demonstrated the deer mouse to be less susceptible to the Montana strain than to other strains of E. multilocularis. This may suggest that the deer mouse is not an important intermediate host in southwestern Montana.

Gerbils and cotton rats have been shown to be highly susceptible to infection with ova of the Alaska and North Dakota strain of E. multilocularis (Sadun et al., 1957; Norman and Kagan, 1961; Kagan et al., 1965). Attempts at infecting gerbils and cotton rats with ova during the current study failed to produce infections. This is also indicative of a strain difference, expecially since 6 cotton rats received their inocula from the same egg pool as the experimental muskrats.

The apparent differences in host specificity of the Montana strain of E. multilocularis may indicate that selection with regard to infectivity and pathogenicity has occurred. Hence, the Montana isolate of E. multilocularis may represent a separate subspecies. Similarly, after detailed experimental studies, Vogel (1957) determined that the Alaskan and European forms of E. multilocularis varied in minor details of structure, host specificity, and pathogenicity. He therefore proposed that the Alaskan form be regarded as a subspecies of the European species (Rausch, 1967). Geographic isolation of a species has been theorized to lead to genetic divergence (Dobzhansky, 1970). Populations of E. multilocularis in Montana are indeed separated from Alaskan populations by a great distance. Also, E. multilocularis occurs throughout a wide geographical range, but the distribution tends to be focal in nature and restricted to areas where the proper conditions exist for survival. This focal

distribution in itself increases genetic isolation, with the migration of the definitive host from one focus of infection to another as the primary means of gene dispersal.

The natural focal distribution allows for operation of the founder principle (Dobzhansky, 1970). The establishment of a new population or focus of infection by a few adult worms carrying only a fraction of the total genetic complement of the parental population leads to a great reduction in genetic variability. In the case where a new focus of infection is established with a limited genetic variability, selection would operate to favor those existing traits and new mutations which enhance the survival of the cestode. Genetic drift and selection may account for the differences in susceptibilities of rodents to the various isolates of E. multilocularis. In southwestern Montana, drift may be tending towards increased infectivity in muskrats and reduced infectivity in other small mammals.

Since foxes and coyotes from Montana are known to harbor E. multilocularis adults, persons coming in contact with these animals are at high risk of becoming exposed to E. multilocularis ova. Thus far there are no records of human cases of alveolar hydatid disease in Montana. From June, 1977 to July, 1981 168 people comprising a high risk group were tested serologically. The test group consisted of trappers, game biologists, fur dealers, and laboratory technicians who came in contact with fox and coyote carcasses. All serologic

tests were negative, indicating there had been no exposure to E. multilocularis ova (Worley et al., unpublished data). Since humans are susceptible to larval infection with E. multilocularis, the lack of human cases in Montana may reflect the host specificity of the Montana isolate of E. multilocularis. The lack of human cases of alveolar hydatid disease also may be due to misdiagnosis of the disease.

The possible existence of a domestic life cycle of E. multilocularis would increase the likelihood of human exposure. Domestic dogs and cats have been reported to be naturally infected with E. multilocularis adults (Rausch and Schiller, 1956; Wobesser, 1971). Experimental infection of domestic cats has proven their susceptibility to E. multilocularis (Rausch and Richards, 1971). The susceptibility of domestic cats to the Montana isolate of E. multilocularis was demonstrated by Eastman and Worley (1979) when infections were obtained in 3 kittens which had been fed viable protoscolices from a naturally infected muskrat. During the present study, 2 adult domestic cats were fed viable protoscolices from a naturally infected muskrat and an experimentally infected deer mouse. In both cases infections did not develop. The natural immunity of adult cats may be sufficient to inhibit infection with the Montana isolate of E. multilocularis.

The maintenance of larval E. multilocularis in the laboratory is routinely performed by intraperitoneal injection into rodent intermediate hosts. During this study, 15 deer mice and 14 cotton rats were inoculated intraperitoneally with cyst material from naturally or experimentally infected rodents. According to the results of Sadun et al. (1957) and Kagan et al. (1965), cotton rats are highly susceptible to infection via intraperitoneal inoculation of cyst material. The successful culturing of E. multilocularis cysts in 4 cotton rats supports the results of previous workers. Deer mice have been difficult to infect with ova of the Montana isolate of E. multilocularis. The results of this study demonstrated that deer mice were refractory to infection via intraperitoneal inoculation with viable cyst material.

Further maintenance of the Montana isolate of E. multilocularis in laboratory animals will enable the pathogenicity and infectivity of the larval cestode to be tested, and also the genetic makeup of the Montana isolate can be compared to other strains of E. multilocularis to determine if subspeciation has occurred. The cultured Montana isolate will also permit testing of the susceptibility of various native Montana carnivores to infection with the adult cestode. Further studies need to be performed with regard to the natural intermediate hosts of E. multilocularis in Montana. Although deer mice

and muskrats in eastern and southwestern Montana, respectively, have been found to be naturally infected with larval E. multilocularis, there is a need for much more information in order to evaluate the roles of these and other small mammals in the maintenance of the life cycle of E. multilocularis in Montana.

SUMMARY

From 1979-1981, 1,245 small mammals, representing 17 species were collected from 7 Montana counties and examined for larval Echinococcus multilocularis. Two naturally infected muskrats were collected from the East Gallatin River, 15 km northwest of Bozeman, Montana. No other species of small mammals were found to be infected. The results of this study support the findings of Eastman and Worley (1979), demonstrating the involvement of the muskrat in the life cycle of E. multilocularis. Southwestern Montana consists of a variety of habitat types which in turn supports a diverse small mammal community. In disjunct areas where suitable habitats support large muskrat populations, the conditions for a fox-muskrat predator-prey relationship exist, thereby facilitating fox-muskrat transmission of E. multilocularis.

Experimentally, the susceptibilities of 2 native rodents, 3 exotic rodents, and one lagomorph were tested. Infections occurred in one of 4 muskrats and one of 31 deer mice. The rate of cyst growth was greater in the muskrat than in the deer mouse, demonstrating that the muskrat was a more suitable intermediate host for E. multilocularis.

Apparent differences in the infectivity and cycle of the Montana isolate indicate that the Montana strain may differ genetically from other North American isolates of E. multilocularis. Genetic

differentiation of E. multilocularis would be enhanced by : 1) geographic isolation which leads to genetic divergence; 2) the natural focal distribution of the cestode reducing gene flow from one population to another; 3) the establishment of a new population or focus of infection by a few individuals which greatly reduces genetic variability. In southwestern Montana, selection may be directed towards increased infectivity of the larval cestode in the muskrat and decreased infectivity in other small mammals.

The failure of 2 adult cats to develop infections with adult cestodes after inoculation with viable protoscolices is contrary to the findings of Eastman and Worley (1979), who successfully infected 3 kittens with adult E. multilocularis. The results of the current study indicate that the natural immunity of adult cats may inhibit infection with the Montana isolate of E. multilocularis.

Culturing of the Montana isolate of E. multilocularis by intraperitoneal inoculation was successful in 4 cotton rats. Attempts to induce larval infection in deer mice, via the intraperitoneal route, were unsuccessful.

APPENDIX

Examination of the viscera of small mammals for larval Echinococcus multilocularis provided the opportunity to obtain information concerning liver parasitism of native Montana small mammals. Three metazoan liver parasites other than E. multilocularis were encountered and identified from the mammals examined. The adult nematode Capillaria hepatica occurred in 18.2% of the 1,245 mammals examined. Larval cestodes of the species Taenia (=Hydatigera) taeniaeformis and Taenia mustelae were found to infect 2.2% and 15.5% of the small mammals respectively.

The prevalences of the 3 parasites in each small mammal species collected are presented in Table III. Capillaria hepatica was the most frequently encountered liver parasite. It has been found to parasitize a wide variety of mammals, including man, although it is most frequently a parasite of rodents (Cheng, 1973). Freeman and Wright (1960) postulated 2 possible routes of infection. One route involves cannibalism of an infected host, followed by the passage of unembryonated eggs in the feces, and then ingestion of embryonated eggs by coprophagy. The second route of infection occurs when a predator devours an infected host, unembryonated eggs are passed with the feces, followed by ingestion of eggs by coprophagy or consumption of contaminated water or vegetation. Cannibalism is a likely source of infection for some rodent species; the data presented in Table III

TABLE III. Prevalences of 3 Metazoan Liver Parasites From Montana Small Mammals.

Mammal species	% Infected		
	Capillaria hepatica	Taenia taeniaeformis	Taenia mustelae
Sorex vagrans	2.0	0	0
S. cinereus	0	0	0
S. palustris	0	0	0
Peromyscus maniculatus	23.8	0	0
Ondatra zibethicus	23.8	4.2	29
Microtus longicaudus	19.0	0	19.0
M. montanus	0	0	0
M. pennsylvanicus	5.3	0	3.2
Clethrionomys gapperi	4.3	0	0
Arvicola richardsoni	0	0	0
Mus musculus	0	0	0
Castor canadensis	4.2	0	4.2
Zapus princeps	7.1	7.1	0
Spermophilus richardsoni	90.0	0	0
Eutamias amoenus	25.0	0	0
Thomomys talpoides	0	0	0
Lepus townsendii	0	0	0

pertaining to Richardson's ground squirrel support this theory. Eighteen of 20 Richardson's ground squirrels collected from one colony were infected with C. hepatica. Ground squirrels are known to be very cannibalistic, and once the parasite infection enters a colony, it is likely to spread rapidly and involve a large percentage of the ground squirrels in the colony. Muskrats maintained a fairly high prevalence of infection and it is possible that their source of infection would be contaminated water.

The strobilocercus of Taenia taeniaeformis was found in 1 of 14 western jumping mice (Zapus princeps) and 27 of 647 muskrats. This tapeworm occurs in the small intestine of various carnivores, but most frequently parasitizes felids (Cheng, 1973). Eggs shed with the feces of the definitive host are ingested by various rodents and lagomorphs and the larvae usually develop in the liver of the intermediate host. McBee (1971) found the prevalence of voles infected with T. taeniaeformis strobilocerci in Gallatin Co., Montana to be correlated closely with large numbers of definitive hosts, primarily domestic cats. He noted that the highest prevalence of infection in voles was from an area just outside of Bozeman, MT. and within easy range of domestic cats, while a rural area several miles away from Bozeman exibited a much lower prevalence of infection. The low prevalence of T. taeniaeformis strobilocercus infection in the mammals examined during this study is probably due to the fact that

the collection sites were in rural areas where there were few domestic cats.

The larva of Taenia mustelae occurred most frequently in muskrats and long-tail voles (Microtus longicaudus), while the prevalences of infection in meadow voles (Microtus pennsylvanicus) and beavers were much lower. The larval stage of T. mustelae is often polycephalic and resembes a coenurus type of tapeworm larva. Eastman (1978) reported 23% of the muskrats examined from southwest Montana to be infected with a coenurus larva. Twenty-nine percent of the muskrats examined from Montana during the present study harbored cestode larvae similar to those reported by Eastman (1978). Measurement of the hooks of the larval cestode and comparison with descriptions by Freeman (1956) and Verster (1969) showed the larval cestode to be Taenia mustelae. The photomicrograph in Fig. 7 shows a portion of a protoscolex with hooks from a section of muskrat liver containing T. mustelae larvae. Freeman (1956) described the hooks as ranging in size from 14-20 μm. The hooks shown in Fig. 8 fall within that range. Freeman also noted that both mono- and polycephalic forms of the larva occurred naturally. He also discovered that both larval forms sometimes occurred in the same intermediate host. Mono- and polycephalic larvae of T. mustelae were found simultaneously in muskrats and meadow voles during this study. T. mustelae, as the name implies, is a parasite of mustelids. Freeman (1956) found mink (Mustela vison)

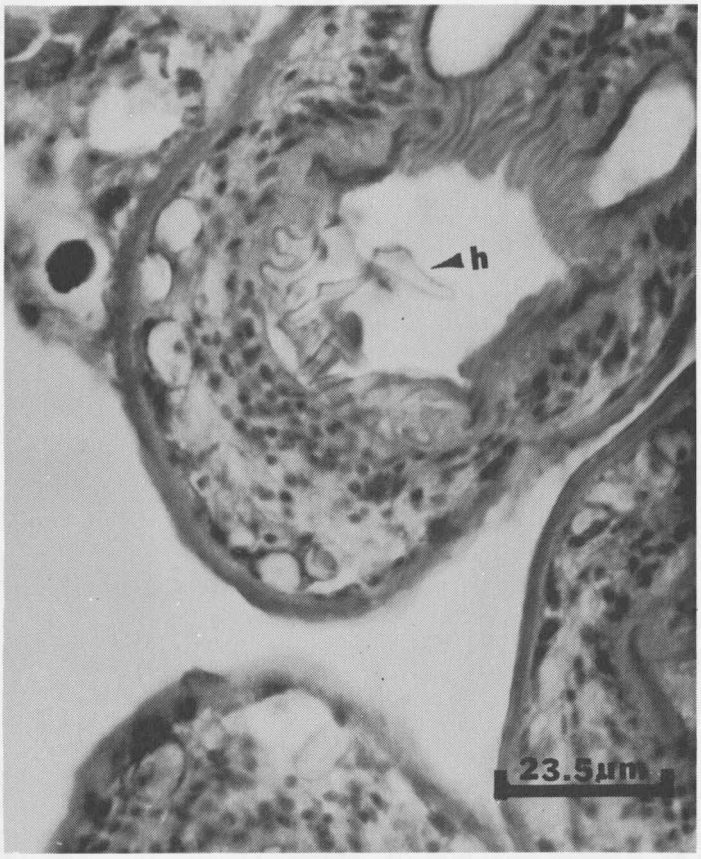

Figure 7. Section through a cyst of T. mustelae showing a protoscolex possessing hooks (h).

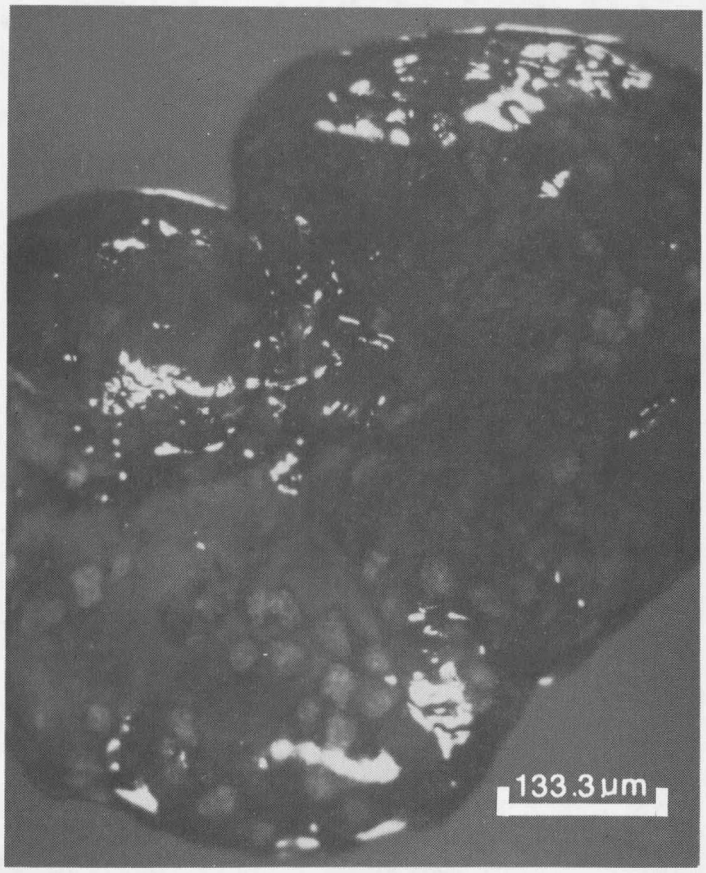

Figure 8. Photomacrograph of a muskrat liver infected with T. mustelae larvae.

and ermine (Mustela erminea) to be common definitive hosts of T. mustelae. Mink are frequent predators of muskrats (Errington, 1954), and the life cycle of T. mustelae in southwestern Montana probably is a muskrat-mink cycle. Muskrats seem to be highly susceptible to infection with T. mustelae larvae since many of the infections consisted of more than 1 larval cyst. A muskrat liver with a hyperinfection of T. mustelae larvae is seen in Fig. 8. Many polycephalic larvae are evident throughout the hepatic parenchyma. The high prevalence of infection and the apparent susceptibility of the muskrat to infection with larval T. mustelae are indicative of the importance of the muskrat in the life cycle of T. mustelae in southwestern Montana.

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