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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by

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

of

MASTER OF SCIENCE

in

Zoology

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Bozeman, Montana

December, 1981

ACKNOWLEDGMENTS

The author wishes to express his appreciation and thanks to Dr. D. E. Worley for his supervision and guidance during this study. Thanks are extended to Dr. R. E. Moore and Dr. L. R. Irby. Appreciation is also extended to M. C. Sterner for technical assistance and aid in small mammal collecting; to Dr. M. W. Hull for aid in surgical procedures; to Merrie Mendenhall for the photography; to Marvin Donahue and other Montana trappers for donating muskrat and beaver carcasses; and to Betty Freeland for typing the manuscript.

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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

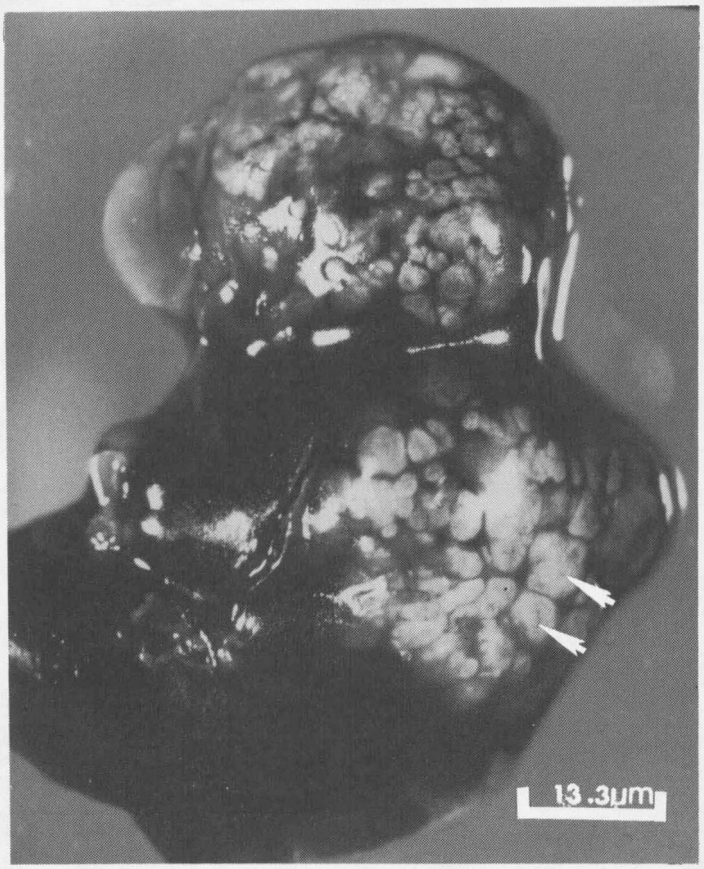

Figure 1. Photomacrograph of a muskrat liver naturally infected with larval E. multilocularis. Arrows (↗) point to daughter cysts.