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


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_. multiloculafis 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.

STATEMENT OF PERMISSION TO COPY

In presenting this thesis in partial fulfillment of the requirements for an advanced degree at Montana State University, I agree that the Library shall make it freely available for inspection. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by my major professor, or, in his absence, by the Director of Libraries. It is understood that any copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Signature Kathy Lee Eastman

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

Approved:

David E. Worley
Chairman, Graduate Committee

David M. Young
Head, Major Department

Henry L. Parsons
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

June, 1978

## ACKNOWLEDGMENTS

The author would like to express her appreciation to Dr. D. E. Worley for his assistance, guidance, and encouragement in this study. Appreciation is also extended to Dr. R. E. Moore, Dr. J. E. Catlin, and to Dr. G. Ford. A special thanks goes to Don Fritts for the photography and to Betty Freeland for typing the manuscript. Recognition also goes to Marvin Donahue for his cooperation in providing muskrat carcasses for study purposes.

TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# 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

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 invade 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 involvement 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 identifing 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 vegatative 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 refactory 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 scolices 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

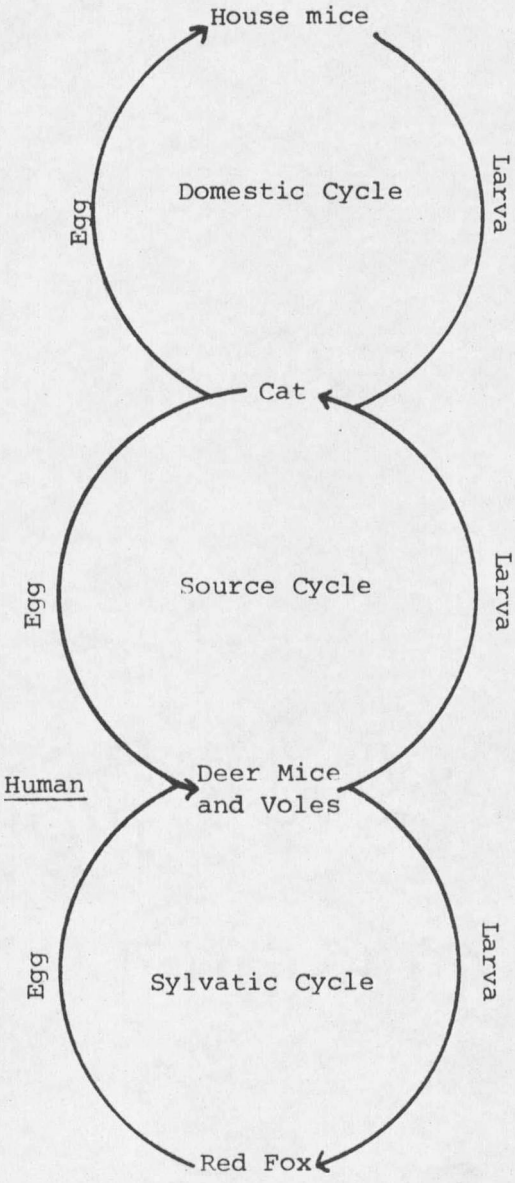


Fig. 1. Life cycle of Echinococcus multilocularis with domestic animal involvement and possible human infections (15).

TABLE I

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

| Animals Examined | Number infected / Number examined |
|---|---|
| Deer Mice (*Peromyscus maniculatus*) | 0/118 |
| Shrews (*Sorex* spp.) | 0/16 |
| Voles (*Microtus* spp.) | 0/16 |
| House mice (*Mus musculus*) | 0/18 |
| Muskrats (*Ondatra zibethicus*) | 2/192 |