Geothermal habitats as sites for year-round transmission of Fasciola hepatica
by Robert Stanley Potts, Jr

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Veterinary Molecular Biology
Montana State University

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Transmission of Fasciola hepatica in western Montana usually occurs during the fall months. However, a reported infection of F. hepatica in a bison herd winter pastured near a thermal site suggested that geothermally influenced habitats may serve as year-round foci of parasite transmission. To investigate this hypothesis, snails were collected and climatological conditions were monitored at four geothermal habitats on a monthly basis from July 1993 to August 1994. Lymnaeid snails were identified and their seasonal population cycles determined. Climatological data were analyzed to determine suitability of thermal habitats as year-round sources of F. hepatica miracidia and metacercaria. Laboratory- reared generations of field-collected lymnaeid snails, originating from thermal and non-thermal habitats, were exposed to F. hepatica miracidia to determine infection rates. Lymnaeid snails were collected from three of four thermal habitats and four species were identified. Lymnaeid snails were recovered at least ten months out of the year at each thermal habitat, with population cycles peaking in March-April and July-September. Review of climatological profiles indicated that temperature and moisture regimes were suitable for F. hepatica development and transmission year-round. Snail species of thermal and non-thermal origins demonstrated significantly different F. hepatica infection rates on a site specific basis.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Date 5 June 1995

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ABSTRACT

Transmission of Fasciola hepatica in western Montana usually occurs during the fall months. However, a reported infection of F. hepatica in a bison herd winter pastured near a thermal site suggested that geothermally influenced habitats may serve as year-round foci of parasite transmission. To investigate this hypothesis, snails were collected and climatological conditions were monitored at four geothermal habitats on a monthly basis from July 1993 to August 1994. Lymnaeid snails were identified and their seasonal population cycles determined. Climatological data were analyzed to determine suitability of thermal habitats as year-round sources of F. hepatica miracidia and metacercaria. Laboratory- reared generations of field-collected lymnaeid snails, originating from thermal and non-thermal habitats, were exposed to F. hepatica miracidia to determine infection rates. Lymnaeid snails were collected from three of four thermal habitats and four species were identified. Lymnaeid snails were recovered at least ten months out of the year at each thermal habitat, with population cycles peaking in March-April and July-September. Review of climatological profiles indicated that temperature and moisture regimes were suitable for F. hepatica development and transmission year-round. Snail species of thermal and non-thermal origins demonstrated significantly different F. hepatica infection rates on a site specific basis.

INTRODUCTION

Three factors determine the development of liver fluke disease caused by members of the Fasciolidae. These include characteristics of the definitive host, the intermediate host and the parasite (Malone and Zukowski, 1992). The definitive host is usually a wild or domestic ungulate but may include other herbivorous animals as well as humans. The snail intermediate hosts for liver flukes belong to the family Lymnaeidae. The principle species of liver fluke of concern to the livestock industry in Montana is Fasciola hepatica although the North American deer fluke, Fascioloides magna, may also be present (Knapp et al., 1992). Parasite transmission is highly complex as each of the components of the Fasciola-Lymnaea system may affect the others and is influenced by the environment. Whereas each component is essential and their biological characteristics affect the incidence of disease, environmental conditions are rate limiting with respect to snail and parasite development in the northwestern United States. The snail intermediate hosts and parasite are sensitive to temperature and moisture and as a result development and transmission of the parasite is usually seasonal in Montana. Additionally, transmission tends to be focal in nature, often occurring in specific microhabitats. Thermal habitats are abundant and widely distributed throughout the state and exhibit many characteristics of microhabitats that favor the occurrence of fasciolosis. The relationship of thermal habitats to the development of liver flukes and the possibility of such areas presenting a year-round microhabitat for transmission is the topic of this study.

Fasciola hepatica

Fasciola hepatica, the common liver fluke, is believed to have been first described in 1379 by Jehan de Brie in France, making it one of the oldest known parasites (Ollerenshaw, 1959). The life history was worked out in 1882 when Lymnaea truncatula was determined to be the intermediate host in Germany (Leuckart, 1882) and England (Thomas, 1883). In North America, Lymnaea (= Galba) bulimoides was the first intermediate host identified for Fasciola hepatica (Shaw and Simms, 1929).

Life Cycle

Fasciola hepatica is a digenetic trematode. The intermediate host is a mollusc and the final host is a vertebrate. The life cycle consists of an alternation of developmental and transport phases, each of which typically result in a variable level of mortality (Ollerenshaw, 1959). Adult flukes occur in the liver and bile duct of the final host, where they have been reported to maintain an infection for as long as 11 years (Price, 1956). Flukes are hermaphroditic and produce thousands of eggs which are deposited in the bile duct and pass to the intestine by way of the common bile duct. Further development to the hatching stage is dependent upon liberation from the feces as well as temperatures of at least 10 C and soil moisture conditions at or exceeding soil water holding capacity (Ollerenshaw, 1959). The time required for the egg to develop to the miracidial stage varies with environmental conditions between two and six weeks. The ciliated miracidia have an active period measured in hours during which time contact and penetration of an intermediate host must occur. Once penetration has occurred the miracidia change into sporocysts. Each sporocyst gives rise to many redia which in turn give rise to daughter redia. Ultimately, daughter redia give rise to cercaria which leave the snail through the mantle. The cercaria are free swimming and eventually encyst on vegetation, losing their tail in the process and becoming metacercaria. Metacercaria are infective to animals which ingest them while grazing. Upon ingestion, the metacercaria excyst in the stomach and bore through the wall of the gut into the abdominal cavity and eventually reach the surface of the liver. The immature flukes then bore into the liver parenchyma, tunneling through and feeding until they reach the bile ducts. Upon arrival, this new generation of adult flukes deposit eggs and the cycle is complete.

Fasciola hepatica in Montana

Until recently, (Knapp et al., 1992) fasciolosis was not considered to be an important disease of domestic livestock in Montana as the cold dry climate was considered to limit the disease to a few of the western counties. Jacobson and Worley (1969) reported detecting fluke ova in 2.4% of 791 samples and noted that infected cattle were primarily distributed in the western region of the state. In 1973, 5% of the livers from Montana slaughter cattle were reported to be condemned because of F. hepatica (Foreyt and Todd, 1976). Recently, however, a study of United States Department of Agriculture (USDA) inspected slaughter plants in Montana reported that liver flukes were present in 17.24% of the 6,032 cattle processed during a 12 month period and that the parasite is widely distributed (Knapp et al., 1992). Subsequently, a survey of beef cow processing facilities in the western United States reported the prevalence rate of F. hepatica to be $19.2 \pm 1.2\%$ (Briskey et al., 1994). The parasite has been reported in 26 of 56 counties in Montana. Infections appear to be heaviest in the Bitterroot Valley area, where 90% of the livers from slaughter cattle were reported to be condemned because of liver fluke (Knapp et al., 1992). Based on studies using tracer sheep, transmission is thought to occur primarily in the autumn as metacercaria are not able to overwinter, rendering pasture essentially parasite free in the spring (Hoover et al., 1984; Knapp and Abrahamsen, 1994).

Lymnaeid Snails

The Lymnaeidae are amphibious snails that belong to the subclass Pulmonata. The family contains over one thousand species (Cruz-Reyes, 1982) and is world-wide in distribution.

Life Cycle

Lymnaeid snails are hermaphroditic, although they may cross fertilize. Eggs are laid in gelatinous capsules and are attached to surrounding substrate. The capsules contain a varying number of eggs ranging from 4 to 180 (Hyman, 1967). The eggs develop into veliger larva within the egg capsule and emerge as young snails anatomically complete except for the reproductive system. Length of development within the egg capsule is temperature dependent. Snails do not develop at temperatures below 9 C or above 37 C. At 9 C the snails hatch from their eggs in about 30 days, at 17-19 C in 17-22 days, and at 25 C in only 8-12 days (Roberts, 1950). Snails typically undergo logarithmic growth with rapid growth in length or diameter at first, then slowing down to a level with little or no growth. Growth commonly ceases after attainment of sexual maturity. Most pulmonates live about one year and die after one spawning (Hyman, 1967).

Ecology

Members of the family Lymnaeidae can be found in many habitat types, including lakes, ponds, ditches, rivers, swamps, and irrigated and non-irrigated pastures. Lymnaeids occur in a wide range of conditions, from sea level to 10,000 feet elevation, from ice waters to hot springs, and in lakes from shallow waters to depths of 250 m (Hyman, 1967). Temperature and moisture have been reported to be the most important factors regarding the development of lymnaeid snails (Ollerenshaw, 1959), but other environmental factors are considered to be important as well. Lymnaeids are generally not associated with rapidly flowing water, however, a moderate flow rate is beneficial (Boray, 1964). A preference of soil type has been reported by several investigators. Ökland (1935), Peters (1938) and Wetzel (1953) each concluded that lymnaeids prefer clay soil. Schmid (1934) suggested that an important factor of soil may be the level of impermeability to water. Several authors have suggested the importance of both soil and water pH. The range of soil pH favored is given variously as 6.2 - 7.2 (Mehl, 1932; Bryant, 1935) and 6 - 9 (Schadin, 1937). Hyman (1967) reported that lymnaeids live in slightly alkaline waters to a maximum of pH 8.5. The alkalinity of the water was due to the presence of calcium carbonate, of which a minimum of 20 mg/l is essential for the well-being of the snails (Boycott, 1936).

Lymnaeids in Montana

The history of lymnaeids in Montana dates back to 1860 when several species were collected from the Missouri River and Hell Gate River (Cooper,1868). Currently, 18 species have been identified (Table 1) and specimens have been found in 29 of 56 counties. Of the 18 species of lymnaeid snails found in the state, 6 have been reported as natural vectors and 5 have been reported as experimental vectors of F. hepatica in various places in the United States (Tables 2 and 3).

Thermal Habitats

There are 96 thermal habitats in the state of Montana with estimated reservoir temperatures ranging from 20 C to 136 C (Sonderegger et al., 1981). The various springs and wells are located in 29 of 56 counties. Thermal waters in Montana are classified according to water temperature as hot, warm or tepid and they represent the largest and most varied group of hot springs in the world (Brues, 1932). Climatic differences relating to temperature and humidity limit the distribution of animals, resulting in regionally distinct fauna (Brues, 1932), but thermal waters present notable exceptions. In Montana, thermal habitats provide opportunities for faunal growth year-round that would not otherwise occur.

Lymnaeids and Thermal Habitats

Many molluscs, including lymnaeid snails, inhabit hot springs in Europe, Iceland and America (Brues, 1932). These snails have been found in waters ranging in temperature up to 45 C. In Montana, Oswald (1979) reported finding lymnaeid snails from the Ringling thermal well in Meagher county and Dunkel et al. (1995) collected several species from thermal habitats throughout the state.

Rationale for the Research

First, Fasciola hepatica is a parasite of considerable economic importance in Montana and throughout the world (Knapp et al., 1992; Boray, 1994). Liver flukes negatively affect the condition of livestock in numerous ways and result in decreased food production and significant economic losses (Chick, 1979; Dargie, 1987). Second, agriculture is the top grossing industry in Montana, resulting in 2.2 billion dollars in cash receipts per year, of which livestock sales accounted for 52.7% in 1993 (Sands and Lund, 1994). Third, the few studies that have been completed suggested that F. hepatica transmission in the northern Rocky Mountain region was seasonal, occurring primarily during the autumn (Hoover et al., 1984; Knapp and Abrahamsen, 1994). However, these studies did not consider the possible effects of microclimates, which Smith and Wilson (1980) and Malone and Zukowski (1992) consider to be important factors in the transmission of F. hepatica. Observation of an outbreak of fasciolosis in a herd of American bison (Bison bison) that had been winter pastured near a thermal habitat (Knapp, personal communication), and a report of clinical fasciolosis in a goat herd in Hot Springs Montana in which parasite transmission occurred in November (Leathers et al., 1982) suggested that transmission could occur year-round in association with thermal habitats. Fourth, thermal habitats are abundant in Montana, occurring in 35 of 56 counties, and their spatial distribution is positively correlated ($p < 0.01$) to the occurrence of liver flukes. Finally, environmental conditions related to temperature and moisture are the most important factors influencing the development of lymnaeid snails and F. hepatica.

Statement of Problem

The purpose of this study was to explore the effects of geothermal habitats on lymnaeid snail populations and liver fluke transmission in Montana. The proposed research was designed to determine the following:

1. the species of lymnaeid snails present at several thermal habitats;

2. the seasonal population dynamics of lymnaeid snails at thermal habitats (population dynamics refers to the number of snails present and the age structure of the population each month over the course of the year);

3. the macro- and microclimatological conditions of several thermal habitats throughout the year and, thereby, indirectly determine their suitability as a year-round source of F. hepatica miracidia and metacercaria; and

4. if snails from thermal and non-thermal habitats differ in susceptibility to F. hepatica.

Snail Species	Collection Site, Date	Reference
Lymnaea bulimoides (Lea)	Missouri river, 1860	Cooper, 1868
L. disidiosa (Say)	Missouri river, 1860	Cooper, 1868
L. humilis (Say)	Missouri river, 1860	Cooper, 1868
L. palustris (Linn)	Hell Gate river, 1860	Cooper, 1868
L. montanensis	Hayes Creek, 1912	Baker, 1913
L. caperata	Winnecook Lake, 1914	Berry, 1916
L. obrussa (Say)	Elk Creek, 1914	Berry, 1916
L. parva	Winnecook Lake, 1914	Berry, 1916
L. binneyi	Madison River, 1924	Taylor, 1952
L. hinkleyi (Say)	Yellowstone Lake, 1935	Taylor, 1952
L. elrodiana (Baker)	St. Mary's Lake, 1960	Russell and Brunson, 1967b
L. stagnalis (Say)	Lake McDonald, 1966	Russell, 1967a
L. dalli	Beaverhead Co, 1989	Dunkel et al., 1995
L. elodes	Gallatin Co., 1989,	Dunkel et al., 1995
L. modicella	Beaverhead Co., 1989	Dunkel et al., 1995
L. techella (Haldeman)	Missouri River, 1990	Knapp, pers. comm., 1993
L. auricularia	Rattlesnake Lake, 1993	Knapp, pers. comm., 1993
L. catascopium	Flathead river, 1993	Knapp, pers. comm., 1993

Table 1. List of lymnaeid snails found in Montana.

Snail Species	**Collection Site**	**Reference**
Lymnaea bulimoides	Eastern Washington	Lang, 1977
L. caperata	Sanders Co., Montana	Knapp, pers. comm., 1993
L. modicella	Eastern Washington	Lang, 1977
L. obrussa	Beaverhead Co., Montana	Knapp, pers. comm., 1993
L. palustris	Eastern Washington	Lang, 1977
L. stagnalis	Eastern Washington	Lang, 1977

Table 2. Species of lymnaeid snails collected in Montana which have previously been found to harbor natural infections of F. hepatica.

Snail Species	**Reference**
Lymnaea modicella	Krull, 1933
L. montanensis	Rowan et al., 1966
L. palustris	Lang, 1977
L. stagnalis	Lang, 1977
L. bulimoides	Foreyt and Todd, 1978

Table 3. Species of lymnaeid snails collected in Montana which have been experimentally infected with F. hepatica.

STUDY SITES

The four thermal habitats represented three geographically distinct regions of the state and were selected based on existing knowledge of the distribution of liver flukes, lymnaeid snails (Knapp et al., 1992) and thermal habitats (Sonderegger and Bergantino, 1981) (Fig.1). Each thermal habitat had previously been visited as part of a state-wide survey for lymnaeid snails (Dunkel et al., 1995).

Green Springs (Hot)

Green Springs (Fig. 2) effluent emanates from a Precambrian Piegan rock formation and Alluvium sediments (Sonderegger et al., 1981) in Sanders Co., Latitude 47D 26M 35S, Longitude 114 D 40M 7S. An analysis of water chemistry is presented in Table 4. The thermal habitat was a marshy depression in the middle of a pasture which was continuously filled by the spring. The area covered about 0.2 ha and was 0.6 meters deep near the center. Cattle were present much of the year and created a mud flat region around the perimeter of the spring. The elevation was 850 meters. Regional land cover was designated as strongly sloping range (8-15%) and the soil had a water holding capacity of 10.7 cm and an average pH of 7.0 (Caprio et al., 1990). The climax vegetation was Subalpine Fir climax forest, with a typical overstory of 50% Subalpine Pine, 35% Douglas Fir, 10% Ponderosa Pine and 5% Engelmann Spruce (Caprio et al., 1990).

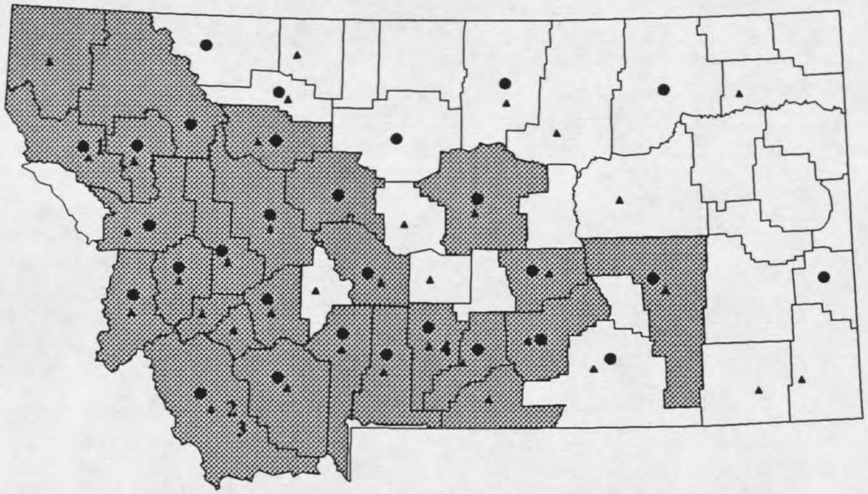

Figure 1. Location of the 4 thermal habitats studied. Distribution of liver flukes (shaded counties), lymnaeid snails (●) and thermal habitats (▴) in Montana by county. (1), Green Springs; (2), New Biltmore Hot Spring; (3), Beaverhead Warm Springs; (4), McLeod Hot Spring.