1971-08-01

A study of snail hosts for Fasciola hepatica in Utah Valley

Robert Michael Briem
Brigham Young University - Provo

A STUDY OF SNAIL HOSTS FOR FASCIOLA HEPATICA IN UTAH VALLEY

A Thesis

Presented to the

Department of Zoology

Brigham Young University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Robert M. Briem

August, 1971

This thesis by Robert M. Briem is accepted in its present form by the Department of Zoology of Brigham Young University as satisfying the thesis requirement for the degree Master of Science.

Typed by Diana Rice

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to my major professor and committee chairman, Dr. Peter A. Nyberg, for his time, direction, and encouragement during my graduate program and the preparation of this manuscript.

Grateful appreciation is extended to the members of my graduate committee, Dr. Lee F. Braithwaite and Dr. J. Keith Rigby for the support and assistance which they so willingly gave during the course of my graduate studies. Grateful acknowledgement is also expressed to Dr. Ferron L. Andersen for his help and encouragement in my graduate endeavors.

I wish to thank Dr. Vasco M. Tanner of the Department of Zoology, Brigham Young University and Dr. James N. Shaw of the Department of Veterinary Medicine, Oregon State University for aid in identification of snails used in this research.

I express my love to my wife for her love, encouragement, and understanding during the course of my graduate program.

Financial aid has been provided by Brigham Young University in the form of teaching assistantships. The Department of Zoology supplied the laboratory space, equipment and supplies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

INTRODUCTION AND REVIEW OF LITERATURE

The Meat Inspection Division of the Bureau of Animal Industry in 1950 reported that 322,647 livers or 2.4% of 13,112,802 cattle slaughtered under United States Federal Inspection were condemned due to liver fluke infections (Price, 1953). This would amount to an annual loss of 3 million dollars in livers alone considering each liver weighed 10 pounds and retailed 65 cents per pound. This figure could conceivably double or triple if additional losses due to animal death, decreased milk production, poor economic utilization of feed or lower livestock prices were considered.

The liver fluke, Fasciola hepatica, is a common parasite of sheep and cattle in Utah Valley. Fox, Andersen and Hoopes (1970), reported that 8.6% of the cattle and 23.5% of the sheep contained eggs of this parasite in their feces. In a necropsy study of 50 sheep and 50 cattle in Utah Valley, Wright (1971) found that 4% of the sheep and 12% of the cattle were infected with F. hepatica. This incidence of infection represents a substantial financial loss to cattle and sheep growers.

The following snails have been identified as possible intermediate hosts of F. hepatica in North America: Lymnaea columella (= Pseudosuccinea columella) (Krull, 1933a), L. bulimoides (= Galba

bulimoides, Galba bulimoides techella) (Shaw and Simms, 1929), L. humilis (= Fossaria modicella, ferruginea, L. obrussa) (Krull, 1933b), L. cubensis (= F. cubensis) (Hoffman, 1930), and L. traskii (Krull, 1934). Lymnaea stagnalis (= L. vulgaris) has been experimentally infected with F. hepatica in Belgium (Berghen, 1964).

A single report exists on the possible snail intermediate host(s) for F. hepatica in the Rocky Mountain area. Krull (1933b) experimentally exposed three species of snails sent to him from Utah (Lymnaea humilis, L. palustris and Succinea avara) to miracidia of F. hepatica. One species, L. humilis became infected and was thus listed as being the probable intermediate host in this area. The present study was done in an effort to find snails naturally infected with F. hepatica. Also an attempt was made to experimentally infect other snail species commonly found in Utah County which might serve as intermediate hosts for this parasite.

MATERIALS AND METHODS

Fluke Eggs

Collection and Cleaning

Gall bladders from sheep and cattle infected with Fasciola hepatica were obtained from a local abattoir. The gall bladders were opened and the contents flushed with distilled water through cheesecloth into 1000 ml beakers. The beakers were filled with distilled water and the suspended material was allowed to settle for one hour. Approximately 700 ml of water was then poured off and the procedure was repeated until the water appeared clear. Finally the excess distilled water was removed and the remaining water and eggs were divided into four equal parts in 250 ml flasks. These were sealed with rubber stoppers and wrapped in aluminum foil.

Incubation

A fluke egg-distilled water mixture in stoppered 250 ml beakers wrapped in aluminum foil was incubated at 27 C in a Thelco Model 2 incubator. This temperature was listed as optimum by Rowan (1956) for embryonation of F. hepatica eggs. Embryonation of the eggs was complete by 9-12 days. The beakers containing embryonated eggs were

stored at 27 C until needed.

Miracidial emergence occurred when embryonated fluke eggs were exposed to light. This was accomplished by removing the aluminum foil from around the flasks containing the egg-water mixture. Miracidia began to emerge from the eggs within one minute after exposure to light.

Snails

Study Area

Study areas were selected after it was determined that fluke eggs were present in fecal samples randomly collected from moist pastures. Surface density of snails per square meter of soil was determined in one study area. Plants commonly associated with snails in that same area were collected and identifications were made by Dr. Stanley L. Welsh of the Department of Botany, Brigham Young University.

Collection

Snails from moist, poorly drained pastures were collected from March 1, 1971 to July 18, 1971. The snails were brought to the laboratory where the majority were crushed to determine if they contained Fasciola hepatica larval stages. Cercarial shedding was induced by placing snails in distilled water contained in 60 mm (outside diameter) petri dishes. The water in each dish was examined 12-24 hours later for the presence of cercaria. Cercarial types were determined according to

descriptions by Cheng (1964) using a Bausch and Lomb Stereozoom microscope equipped with 10 X oculars and a variable power pod with 0.7 X to 3 X gradations. A 2 X supplimentary lens attachment was used for more detailed examinations of larvae.

Identification

Snails collected in study areas were brought to our laboratory and tentatively identified. Confirmation of species was by Dr. Vasco M. Tanner of the Department of Zoology, Brigham Young University and Dr. James N. Shaw of the Department of Veterinary Medicine, Oregon State University.

Laboratory Rearing and Maintenance

Stock cultures of snails were reared in 10 X 4 X 4 inch plastic vessels covered with acrylic plastic sheets. Each culture vessel contained 600 ml of well water which was changed several times each week. Well water was used since chlorinated water is lethal to snails reared in the laboratory (Shaw, personal communication). For the duration of all experiments the water temperature was maintained at 26-27 C in a thermostatically controlled environmental chamber. All snails were fed lettuce.

Experimental Infections

Thirty to fifty mature field collected snails and variable numbers of laboratory reared snails of each species were put into individual 60 mm petri dishes and exposed to 3-5 miracidia each. Fifty mature and a variable number of immature snails of each species were subjected to mass exposure of miracidia in the laboratory. After 24 hours the snails were transferred to glass bowls containing 800 ml of well water and were maintained at 26-27 C. The frequency of water changes and feeding was the same as in the stock cultures. Unexposed control groups were kept for each species of snails.

RESULTS

Field Studies

Snail Ecology

Snails were collected from pastures where cattle infected with F. hepatica were grazing. In these study areas the incidence of F. hepatica infections in cattle as determined by random fecal sampling was variable, and ranged from 1-2% to 36%.

The average soil surface density of Lymnaea humilis in one study area was found to be 1040 sanils per square meter. Plants closely associated with L. humilis were the following: Juncus balticus, Rorippa nasturtium-aquaticum, Berula erecta, Veronica americana and Equesetum kansanum. These plants were usually growing in or very near to water adjoining mud flat areas.

Laboratory Studies

Snail Identification

The following eight species of snails were found in pastures where animals infected with F. hepatica were grazing: Amnicola limosa, Aplexa microstriata, Gyralus vermicularis, Helisoma trivolvis binneyi,

L. humilis, L. stagnalis wasatchensis, Physa ampullacea and Succinea avara (Fig. 1).

Snail Culture

Approximately 50-100 adult snails of each species were collected and reared in the laboratory. Depending on their size, 25-50 snails were reared in each plastic container. All of the snails with the exception of Amnicola limosa layed eggs within eight weeks after collection. Egg masses were carefully removed from the bottom and sides of the culture containers and were transferred to identical containers according to species. Egg masses and snails were maintained at 26-27 C for the duration of the experiment.

Mortality of laboratory reared juvenile snails was less than 20% whereas those for adults were much more variable (Table 1). Lymnaea humilis was the most difficult adult snail to rear. When reared as the other snails, L. humilis adults would usually die within one week after collection. Temperature did not seem to be the primary limiting factor, but instead the degree of moisture. Mud from areas where L. humilis was found was put into the culture containers. Snails thrived in this moist mud habitat, but if the mud became covered with water the snails died as before.

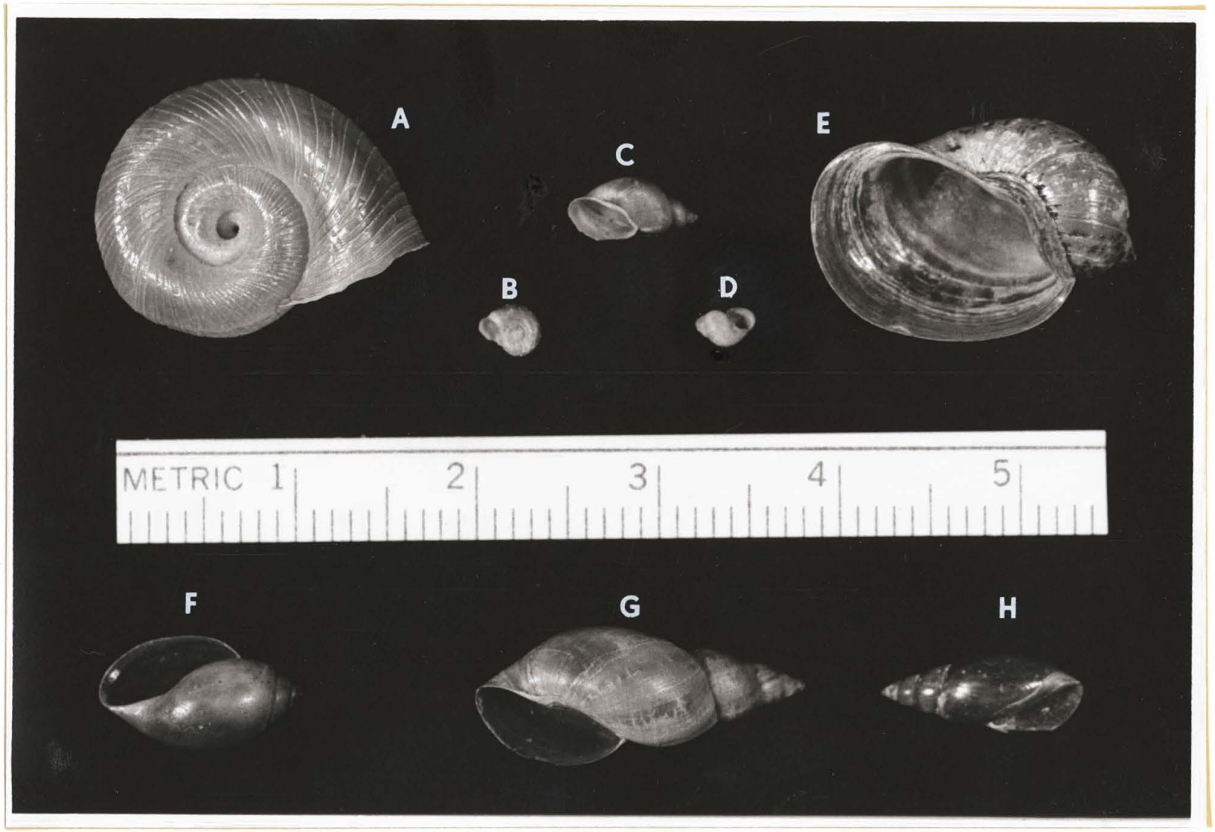

Fig. 1. Snails experimentally exposed to Fasciola hepatica. A, Helisoma trivolvis binneyi. B, Gyraulus vermicularis. C, Lymnaea humilis. D, Amnicola limosa. E, Succinea avara. F. Physa ampullacea. G, L. stagnalis wasatchensis. H, Aplexa microstriata. (X 2.5).

Table 1. In vitro survival of juvenile snails hatched from eggs and of adult snails collected in the field.

Snails	Juveniles	Adults
Amnicola limosa	*	90%
Aplexa microstriata	Greater than 80%	30%
Gyraulus vermicularis	" " "	80%
Helisoma trivolvis binneyi	" " "	95%
Lymnaea humilis	" " "	0%
Lymnaea stagnalis wasatchensis	" " "	75%
Physa ampullacea	" " "	95%
Succinea avara	" " "	80%

*No eggs layed.

Natural Infections

Snails from pastures where infected animals were grazing were collected from March 1 to July 18, 1971 and at least 1000 of each species were crushed to determine if they harbored larval stages of F. hepatica. Snails naturally infected with F. hepatica were not found, however, other fluke species were frequently encountered.

Three of the eight snail species studied were infected with cercaria other than F. hepatica. The following cercarial types in sequence of highest incidence were: leptocercous cercaria with and without eye spots (Physa ampullacea, Lymnaea stagnalis wasatchensis and Amnicola limosa), two furcocercous cercaria without eye spots (P. ampullacea and L. stagnalis wasatchensis) and one furcocercous cercaria with eye spots (P. ampullacea and L. stagnalis wasatchensis), (Figs. 4-8).

Experimental Infections

Fluke eggs incubated in distilled water at 26-27 C were completely embryonated 9-10 days later. Best hatching results were obtained when periodic changes of distilled water were made during the embryonation period. When this procedure was followed 90-95% of the eggs embryonated and hatched.

All seven species of juvenile and eight species of adult snails

listed in Table I, were exposed experimentally to F. hepatica. However, only one, L. humilis, was successfully penetrated by miracidia. Miracidial penetration of L. humilis was complete by 65 minutes after exposure. All other snail species experienced only partial penetration by miracidia. In these instances penetration never occurred past the eye spots and the miracidia eventually became detached from the snail and died.

Redia developed only in Lymnaea humilis and these were first observed 28 days after exposure to F. hepatica miracidia (Fig. 2). Specimens of L. humilis juveniles, periodically crushed to check on fluke development, were found to contain large well developed redia containing cercaria 47 days following exposure to F. hepatica (Fig. 3). Seven percent of the L. humilis juveniles experimentally exposed to F. hepatica, became infected.

Fig. 2. Redia from Lymnaea humilis 28 days after exposure to Fasciola hepatica.

Fig. 3. Mature redia and cercariae of Fasciola hepatica.

DISCUSSION

The results of this research are similar to those obtained by Krull (1933b) in that Lymnaea humilis is the snail host most probably involved in the life cycle of Fasciola hepatica in Utah. Even though six species of snails in this study were different from those in Krull's (1933b) work, none of them were found naturally infected and none became experimentally infected. Lymnaea stagnalis has been experimentally infected in Europe, however, the subspecies, L. stagnalis wasatchensis, used in this study, was not penetrated when experimentally exposed to miracidia.

Due to the high incidence of fascioliasis in Utah Valley (Fox, et al. 1970; Wright, 1971) one might expect to readily find snail species naturally infected with larval stages of F. hepatica. For obscure reasons, however, snails naturally infected with this parasite are extremely difficult to find, and few published accounts of observed natural infections exist (Shaw and Simms, 1929; Sinitsin, 1929). Shaw (personal communication) has reported that in over 30 years of liver fluke research in Oregon, naturally infected snails have been found in only two localities. In all instances the natural infections occurred in snails on pastures in which 100% of the sheep were infected with liver flukes. Also, thousands of snails in Montana were examined for natural infection of

F. hepatica (Marquardt, personal communication), but none were found.

One factor that might be associated with infection of snails is irrigation practices in Utah. This is known to have a significant effect on snail dispersal. For example, snail density may be extremely high one week in a pasture where infected animals are grazing. When a rancher irrigates his pasture, the majority of snails might be carried to an entirely different area by the irrigation water. The above procedures practiced in Utah are contrasted to those in Oregon where natural infections have been found and irrigation is not practiced. Wet environmental conditions characteristic of western Oregon could be conducive to greater percentages of naturally infected snails by allowing snails to remain for extended periods in close association with fecal material from infected animals.

Lymnaea humilis usually selects a mud flat, muddy beach or small pool bordered with moss and debris as a habitat, rarely living in large bodies of stagnant or moving water (McCraw, 1959). In times of drought or when irrigation water recedes the snails burrow into the soft mud to avoid desiccation and emerge again with the return of water. This is different from behavior of some of the other snails studied in that they fail to protect themselves by burrowing into the mud and thus die in times of drought. Also, L. humilis is found concentrated where livestock drink from poorly drained areas. This is contrasted to other species which are often found in larger bodies of water such as ponds

or streams -- habitats not as conducive to maintenance of snail infections.

Since fascioliasis is a problem of economic importance in Utah Valley control measures should be implemented. Snail control can be accomplished in at least two ways: (1) drainage of marshy grazing areas or (2) by chemical treatment (such as copper sulfate) of snail infested areas (Knapp, 1964). One cattle rancher in Springville, Utah, (Sumsion, personal communication) applied copper sulfate to his pastures in 1969 where abundant Lymnaea humilis occurred. Cattle sent to slaughter the following year did not have new infections of F. hepatica.

SUMMARY

Eight snail species were used in this study. None were found to harbor natural infections of Fasciola hepatica larval stages, however, three species were infected with other larval flukes.

When experimentally exposed to F. hepatica miracidia, one species, Lymnaea humilis, became infected and developed larval stages. This is in agreement with Krull's (1933b) findings and suggests that L. humilis is a possible intermediate host for F. hepatica in Utah Valley. High incidence of this snail species in association with F. hepatica infected animals is additional evidence that it might be a natural intermediate host. This must be confirmed, however, by finding naturally infected snails.

LITERATURE CITED

Berghen, P. 1964. Some Lymnaeidae as intermediate hosts of Fasciola hepatica in Belgium. Exp. Parasit., 15:118-124.

Cheng, T. C. 1964. The biology of animal parasites. W. B. Saunders Co., Philadelphia.

Fox, J. C., F. L. Andersen and K. H. Hoopes. 1970. A survey of helminth parasites of cattle and sheep in Utah Valley. Great Basin Nat., 30:131-145.

Hoffman, W. A. 1930. The intermediate host of Fasciola hepatica in Puerto Rico. Puerto Rico J. Pub. Health and Trop. Med., 6:89-90.

Knapp, S. E. 1964. 1964 Sheep and wool day. Oregon State Univ. Agr. Exp. Sta., Special Report 182.

Krull, W. H. 1933a. New snail Pseudosuccinea columella (Say) as a potentially important intermediate host extending the range of Fasciola hepatica Linn. J. Wash. Acad. Sci., 23:389-391.

Krull, W. H. 1933b. New snail and hosts for Fasciola hepatica Linn. J. Parasit., 20:107-108.

Krull, W. H. 1934. The intermediate hosts of Fasciola hepatica and Fascioloides magna in the United States. North Am. Vet., 15:13-17.

Marquardt, W. C. Professor, Department of Zoology, Colorado State University. Personal communication, April, 1971.

McCraw, B. M. 1959. The ecology of the snail, Lymnaea humilis Say. Trans. Am. Micro. Soc., 78:101-121.

Price, E. W. 1953. The fluke situation in American ruminants. J. Parasit., 39:119-134.

Rowan, W. B. 1956. The mode of hatching of the egg of Fasciola hepatica. Exp. Parasit., 5:118-137.

Shaw, J. N. Professor, Department of Veterinary Medicine, Oregon State University. Personal communication, July 1971.

Shaw, J. N., and B. T. Simms. 1929. Galba bulimoides Lea an intermediate host of Fasciola hepatica in Oregon. Science 69:357.

Sinitsin, D. F. 1929. Rediae and cercaria of Fasciola hepatica found in water snails, Galba bulimoides techella. J. Parasit., 15: 222.

Sumsion, H. Rancher. Personal communication, June 1971.

Wright, P. D. 1971. Post-mortem examination of cattle and sheep for parasitic helminths. Unpublished master's thesis, Department of Zoology, Brigham Young University, Provo, Utah.

APPENDIX

Figs. 4-7. 4. Furcocercous cercaria without eye spots, (X 100). 5. Furcocercous cercaria without eye spots, (X 100). 6. Furcocercous cercaria with eye spots, (X 100). 7. Leptocercous cercaria without eye spots, (X 63).

Fig. 8. Leptocercous cercaria with eye spots. (X 63).

A STUDY OF SNAIL HOSTS FOR FASCIOLA HEPATICA IN UTAH VALLEY

Robert M. Briem

Department of Zoology

M.S. Degree, August 1971

ABSTRACT

Although infections with Fasciola hepatica in sheep and cattle are extremely common in Utah Valley, the natural intermediate host(s) have not been determined. In an attempt to find possible intermediate hosts for this parasite, a study was conducted using the following snail species: Amnicola limosa, Aplexa microstriata, Gyraulus vermicularis, Helisoma trivolvis binneyi, Lymnaea humilis, L. stagnalis wasatchensis, Physa ampullacea and Succinea avara.

Snails of each species were collected in pastures where cattle known to be infected with F. hepatica were grazing. A minimum of 1000 snails of each species were crushed and examined for F. hepatica larval stages. No snails were found naturally infected with this fluke species, however, several cercarial types of other flukes were observed.

Seven species of laboratory reared snails and eight species of field collected adult snails were experimentally exposed to F. hepatica miracidia. One species, L. humilis, became infected. This suggests that it is an intermediate host for F. hepatica in Utah Valley.

VITA

Robert M. Briem