Ankara Üniversitesi
VETERİNER FAKÜLTESİ DERGİSİ
A. Ü. Veteriner Fakültesi tarafından üç ayda bir neşredilir
Cilt : VI 1959 No.: 3-4

STUDIES ON THE BIOLOGY OF FASCIOLA HEPATICA IN TURKEY

Nevzat GÜRALP and B. T. SİMMS

The trematodes that attack the liver are one of the more serious groups of parasites of domesticated ruminants. At least one species occurs in nearly every important meat producing area in the world. Turkey is unfortunate in having its ruminants, and occasionally other herbivora, infested with three of them; namely, **Fasciola hepatica, Fasciola gigantica,** and **Dicrocoelium dendriticum.** A fourth species, **Fascioloides magna,** which is very probably of American origin, is known to be present in Europe and is probably spreading. It seems possible that it may invade Turkey in the future. Both **F. hepatica** and **D. dendriticum** are widely distributed in Turkey; **F. gigantica** is somewhat less so.

F. hepatica is of special interest to parasitologists both because of its serious economic importance and because it was the first of the trematode parasites to have its life cycle worked out. Leukart of Germany and Thomas of England, working independently, discovered in 1882 that the mature form of this parasite lives in the bile ducts and gall bladders of mammals, specially ruminants, while the immature stages occur in pond snails. They found a single fluke may produce very many ova and that the parthenitae multiply in the snail host.

The work reported in this paper was planned and carried out to develop additional information concerning this trematode and its snail host in the hope that better methods of control and eradication in Turkey may finally be worked out. Much of it is similar to work done by previous investigators. (See References) This work included collection of snails from several areas in Turkey, observations of such snails under both natural and laboratory conditions, collection and hatching of **F. hepatica** ova, exposure of snails to miracidia, studies of the development of rediae and cercariae in favorable snail hosts,

infestation of experimental animals, and observations of developing **F hepatica** in such animals.

Materials and Methods

1. **Collecting and maintaining snails.** Field trips were made during all seasons of the year to search for fresh-water snails, to observe in their natural habitat any that were found, and to collect specimens for laboratory study. Since it is known that the different snail hosts for **F. hepatica** usually live in shallow water that is either still or running very slowly, special attention was paid to water of this type. When there was opportunity, land snails were collected although there was no reason to believe any of them are natural hosts of **F. hepatica.** When snails were found, representative specimens were put in glass jars or vials along with water from the same source and taken to the laboratory. If collections were made at points more than a day's journey from the laboratory, about 10 or 12 snails of each lot were put in 70 % alcohol for later identification. Each lot collected was given an identifying number. A lot number included only snails of a single species collected the same day and from the same place. Owners of sheep or cattle that grazed the areas that were searched for snails were asked if their animals were infected with flukes.

Snails were kept in the laboratory in glass containers such as petri dishes, cylindrical glass bowls (yogurt bowls about 7 cm high and 10 cm wide, each with a capacity of about half a liter) and rectangular glass jars. In most instances each lot was divided into groups of about 10 each and each group was put in a separate yogurt bowl. Water from the Ankara water system was placed in each bowl to a depth of about one cm. Both green and dead grass leaves were put in the bowls as food. Mud from the place where the snails were collected was put in some bowls along with the grass leaves. Both oatmeal and one of the prepared cereal breakfast foods were given to a few lots. Water was usually changed in each bowl at about semiweekly intervals although there were many exceptions to this. Food was added whenever it seemed necessary, sometimes at intervals of as long as five weeks. The bowls containing strictly aquatic species were frequently left uncovered. Others were covered with inverted petri dish covers, pieces of cardboard, or by setting similar bowls on top of them.

The bowls and jars containing snails were kept on a table in a laboratory room. Some were so placed that the afternoon sun shone on them; others were never in direct sunlight. Room temperature varied from about 10° C. to about 25° C. Water was added as needed. If the bowls became quite soiled with excrement they were cleaned.

2. **Natural infection of snails.** Snails were checked for natural infection by (a) crushing living or recently dead snails and examining them microscopically for rediae or cercariae and (b) holding them in the laboratory for varying periods up to more than 100 days and examining both the water in which they were held for swimming cercariae and the walls of their bowls for encysted cercariae. When each lot was discarded some, but usually not all, of the live snails remaining were crushed and examined for rediae and cercariae.

3. **Collecting ova, hatching them, and exposing snails to miracidia.** **F. hepatica** ova were collected from gall bladders of infected sheep at the local abattoir. They were washed in several changes of water until there was no color of bile left, put in a stoppered glass bottle and stored in a refrigerator. About two weeks before miracidia were needed some of the ova were placed in a covered petri dish and held at room temperature.

When the ova were found by microscopic examination to contain mature, moving miracidia, they were again placed in a refrigerator. On days when miracidia were needed for experiments some of the ova were put in a glass container such as a test tube or a stendor dish and set in direct sunlight. Most of the snails exposed to miracidia under laboratory conditions were from.3 to.6 cm long. They were usually exposed in small stendor dishes. A few drops of water from a dish which contained freshly hatched, active miracidia were placed in a stendor, enough water was added to almost cover the snails to be exposed, and one to three snails were put in the dish. After a few seconds the snails were observed with a dissecting microscope usually at about 30-75X magnification. When one to four miracidia were seen attached to a snail, it was picked up with dissecting forceps and put in another dish containing water. In most instances the snails were examined a second time after a few minutes to determine if the miracidia were actually penetrating the snail.

4. **Development of parthenitae in snails.** Exposed snails were kept in either petri dishes or yogurt bowls and fed the same diet they had eaten before exposure. In some instances two or three snails of an exposed lot were crushed at the 20th to 35th day and examined for developing parthenitae. Nearly all exposed snails that were not crushed for examination were held either until they died or until at least 75 days following exposure. Most of those dying more than 20 days after exposure were crushed and examined for rediae and cercariae. Most of those living until the end of the observation period were crushed and examined. Experimental animals were exposed by scraping metacercariae from the walls of glass containers on which they had

encysted, picking them up along with some water with a medicine dropper, and emptying the dropper on the base of the tongue. If any remained on the walls of the dropper, additional water was used to wash them out. Immediately after the metacercariae were given, three or four droppers of water were placed in the same place on the tongue.

In most instances no serious difficulty was encountered in introducing the metacercariae by this technique.

Results

1. **Snails collected.** Four species of fresh - water snails namely **Lymnaea truncatula,** * **Lymnaea pereger, Succinea** species, and **Gyraulus hebraicus** were collected. Special attention was paid to **L. truncatula** and **L. pereger** because these two species have been reported as being intermediate hosts of **F. hepatica.**

L. truncatula was found to be widespread on the Anatolian Plateau. Specimens of this species were collected in every area but not in every spot where searches were made. They were found near Ciyir village about 20 kilometers south of Kizilcahamam, near Saray village about 20 kilometers from Ankara, near Hasanoglan about 30 kilometers from Ankara, near Erif village about 10 kilometers from Haymana, about 50 kilometers north of Çorum along the road to Samsun, about 110 kilometers beyond Çorum along the Samsun road, about 30 kilometers beyond Bala along the Kayseri road, near Akköy village in Ürgüp district at Tekir, Pozantı district near summit of Toros mountains at elevation of about 1200 meters, at Torun in Kırıkhan district, along Bursa - Karacabey highway about 30 Km, from Bursa, at Kayı village between Kütahya and Tavshanlı, at Ahmet Oluğu village between Kütahya and Eskişehir at Bursa Eskişehir highway, at Mudanya Bursa and Mudanya Tirilye highways. In all instances these snails were found either in shallow, clear, still or slow-moving water that contained vegetation or near such water on very moist soil, or in water troughs containing considerable quantities of algae. Collections were made every month of the year. On one occasion when the soil was frozen no snails were seen on the surface, but when some of the frozen mass was placed in water in a pail and thawed, many snails appeared at the water line on the side of the pail. Most of these snails were actually in water usually not more then 10 cm. below the surface when found.

(*) The authors wish to thank Dr. J. P. E. Morrison, Associate Curator, Division of Mollusks, Smithsonian Institution, United States National Museum, Washington D. C., for identifying snail specimens.

Some were on moist soil; a few were on plants. Cattle and horse tracks containing water were a favorite habitat.

They varied in length from about.1 to about 1 cm. All sizes were found at all seasons of the year.

They apparently did not like conditions in the laboratory when they were first brought in from the field as many of them crawled out of the water and even out of the bowls unless they were covered. Once out of the water and on dry glass they estivated. No estivating snail was ever observed to become active again unless it was placed in water.

Snails held in the laboratory grew rather slowly. Records of growth were kept on several. The following is typical: 10 snails from Lot 46 were measured January 3, 1959 and put in a bowl with the usual food. Their average length was.2 cm with extremes of.14 and.28. Twenty-one days later their average length was.28 cm with extremes of.17 and.45 cm. Egg masses were deposited every season of the year but snails collected in spring were more active in egg production than were those collected during other seasons. Eggs hatched if kept wet. Young snails grew satisfactorily, but mortality among them was high.

Snails of this species did not have as high livability in the laboratory as did some other species. Insofar as could be determined by observations and keeping mortality records no destructive transmissible disease ever appeared among them.

L. pereger was found to be almost as widespread on the Anatolian Plateau as was **L. truncatula.** Specimens were collected at most of the places where **L. truncatula** were found, but if one species was quite abundant the other was usually scarce. **L. pereger** was found often in still water or small ponds with very little water moving out of them. This species was almost never seen on mud or wet loam. In a few instances specimens were collected from slightly muddy sluggish streams. Water filled tracks of horses and cattle often contained these snails.

They lived well in the laboratory eating partly decayed grass leaves and showing little tendency to crawl out of uncovered bowls. Some lots produced eggs and some of these hatched and developed. A second generation was grown in one bowl. No special efforts were made to maintain a colony indefinitely.

Collections of this species were made every month except January.

Specimens of **Succinea** species were collected only one time and only four of them were found. This collection was made near Saray village about 20 kilometers from Ankara. They were in clear, very slug-

gish, very shallow water. These specimens lived well under laboratory conditions.

Gyraulus hebraicus was found to be quite widespread although in most instances only a few specimens were found in any one place. An exception was a shallow, clear, shaded pool near Kizilcahamam. A great many of these snails were feeding on tree leaves in this pool. A high percentage of them were very small, less than.2 cm in diameter. These snails lived well and grew satisfactorily under laboratory conditions.

2. **Natural infection of snails** with **F. hepatica** parthenitae was found in only one species viz **L. truncatula.** Cercaria escaped from snails in three different lots of this species. All of these lots, Nos. 46, 48, and 49, were collected at the same place near Kizilcahamam on January 1, January 25, and March 13, 1959 respectively. Snails from each of these lots were held in groups of about 10 each in bowls. None of these groups was exposed artificially to miracidia. Metacercariae were found on the walls of some of the jars containing snails from each lot. Infection experiments with metacercariae from Lot 46, using guinea pigs as definitive hosts, resulted in the development of typical **F. hepatica** in the livers.

More than 200 specimens of **L. pereger** were held in bowls in the laboratory for from 30 to 90 days. Examinations of these bowls for encysted cercariae, made at frequent intervals, were always negative. Two types of cercariae, neither of which encysted, escaped from some snails of this species. Attempts were not made to find definitive hosts of these.

3. **Hatching F. hepatica ova and infecting snails.** When ova containing mature miracidia were exposed to sunlight, miracidia began emerging in less than five minutes. Being phototropic they collected principally at the side of the container which was nearest the light.

When freshly hatched miracidia were put in a dish with either **L. truncatula** or **L. pereger,** they immediately became more active, swimming more rapidly and circling around the snails or near any trail a snail had left in crawling. Some of the miracidia attacked snails within five to ten seconds after exposure. Many of them changed positions of attack, some up to several times. Some attacked for a few seconds up to more than five minutes, left the snail, and did not attack again. Others became permanently attached after one or more attacks, many within a minute or less of exposure.

Some snails were attacked by large numbers of miracidia within

a few minutes of exposure while others of the same species exposed at the same time were attacked by only a few. No reason for this difference was found. Miracidia were very active for the first few minutes after they became attached to a snail of either of these species; they bent rapidly back and forth with the anterior extremity gradually penetrating the snail host. After a few minutes the attached miracidia became less active. Almost no movement was seen after 30 minutes of attachment. Miracidia penetrated the snail rather slowly. After about two hours about half the parasite was still visible. In three to four hours penetration was completed.

Miracidia attacked and penetrated any exposed part of the snail host. Those attached to the tentacles, the mantle, and the foot were most easily seen. Some miracidia attached themselves to the shell of the snail, remaining in this position for as long as a minute.

When both **L. truncatula** and **L. pereger** were placed in a dish with miracidia at the same time, the miracidia seemed to swarm around the **L. truncatula** species in greater numbers than they did around **L. pereger.** Miracidia did not attach themselves for more than a few seconds to specimens of either **Succinea** species or **G. hebraicus.**

In some instances exposed specimens of lots of **L. truncatula** were marked with nail polish and held in bowls with unexposed snails from the same lots. Even when exposure was heavy both the growth rate and the livability of the two groups were comparable. In some of these experiments mortality was high in both groups. Only a few exposed **L. pereger** were held in jars with unexposed specimens of the same species. Results indicated the parasites might be injurious to this species as they seemed somewhat less active for two of three days following exposure.

Beginning at about the 25 th day following exposure snails of both species were crushed and examined for developing rediae. In no instance was a specimen of **L. pereger** found infected. Developing rediae were found regularly in exposed **L. truncatula.** Up to more than a hundred were counted in a single snail. Cercariae escaped from this species as early as the 42nd day following exposure. It was more usual, however, for them to escape for the first time at the 55th to 70th day.

The longest period during which cercariae were observed to escape from an infected snail was 114 days. This was in a snail of lot 41-C, collected 29.10.1958, exposed 19.11.1958 and held under observation. Cercariae were first seen 22.2.1959 or 95 days after exposure. They continued to escape until 15.6.1959 when the snail died.

Autopsies of snails which had discharged cercariae invariably re-

sulted in finding parthenitae. This indicates that infected snails, kept under conditions existing in our laboratory, do not recover.

More than 90% of the metacercariae observed were encysted on the walls of the glass bowls. A few were on blades of grass in the bowls and an occasional one was on the bottom of a bowl.

It was quite unusual to observe swimming or encysting cercariae even when bowls containing discharging snails were examined two to five or more times per day. Counts of encysted cercariae made at about 8:30 a.m. and 4:30 p.m. revealed the fact that much more than two thirds of the total number of cercariae discharged each 24 hours escaped from their snail hosts between 4:30 p. m. and 8:30 a. m.

Cercariae showed a marked tendency to encyst in clusters on the glass and usually within less than.4 cm of the surface of the water. Preliminary observations failed to associate the location of the clusters with any factor such as light, heat, etc.

Very recently - encysted cercariae were a pearl white color. As they aged they became yellow to yellow-brown. The age of metacercariae could be judged roughly by their color.

Typical lesions of **F. hepatica** infection were found in guinea pigs and rabbits that either died (probably from heavy infestation) or were killed following experimental exposure to metacercariae. In all instances **F. hepatica** were present in their livers. The following protocols are typical.

Guinea pig 46-4-2 — 28 February 1959. Given 27 metacercariae from naturally infected snails in Bowl 46-4-N.

13 April 1959. Dead. 14 immature **F. hepatica** up to 1.2 cm long when moving in warm.85% Na Cl solution recovered from bile ducts. Ascites with fluid blood tinged. Surface of liver very much roughened. Necrotic areas in liver with hemorrhagic borders. Croupous membrane covering the surfaces of these necrotic masses.

Rabbit 46-C-1 — 16 March 1959. Given 30 metacercariae from experimentally infected snails from Bowl 46-C-1.

5 May 1959. Ova typical of **F. hepatica** voided in feces.

6 may 1959. Mature **F. hepatica** found in bile ducts at autopsy.

Discussion

Since rainfall on much of the Anatolian Plateau is only about 30-35 cm per annum a large percentage of the land of the area is too dry to support the fresh-water snails that are intermediate hosts of **F. hepatica.** But spring-fed watering troughs with overlow throughout the year, some naturally swampy areas, and some irrigated fields and pastures provide habitats somewhat limited in size that are very satisfactory breeding places for these snails.

Carrier animals on the Anatolian Plateau spend much of their time grazing over areas that are not adapted to completion of the life cycle of the host snail. They usually do not, then, become as heavily infected as do many grazing animals in areas where rainfall is heavier and a much greater proportion of the grazing land furnishes satisfactory habitat for the host snail. In turn the snails, being exposed to excrement not overburdened with fluke ova, apparently do not usually develop the high percentage of infection seen in some other grazing areas.

The constant presence of water and wet soil just below watering troughs provides such favorable conditions that snails living in such places do not necessarily estivate or become dormant. Infested snails may, then, discharge cercariae during the dry months.

It seems possible that living under conditions existing in these areas has not been accompanied by any natural selection of individuals that estivated most easily. It may be, then, that Turkish snails of this species do not estivate as regularly and easily as do other snails of the species whose habitat has, for many generations, become dry during some seasons of the year.

The occurrence of well developed parthenitae in host snails in March shows that infection overwintered in them. With the first warm spring days, then, cercariae may escape and encyst on vegetation that will be eaten by a susceptible animal.

A relatively small percentage of grazing land in most areas is all that needs to be treated with a molluskicide. This is far different from the situation in some other fluke-infested countries. Many of the wet areas that are favorable habitats for snails are quite well isolated. Once all snails are eradicated from such an area it seems possible it might remain free for many years.

Since herbivora infested with **F. hepatica** show little tendency toward spontaneous recovery grazing grounds may be sources of miracidia for many years if untreated livestock graze on them. Any control or eradication program should, then, include both the destruction of

snail hosts and treatment of all susceptible livestock with some efficient drug.

Conditions in Turkey seem almost ideal for the continued association of **F. hepatica**, its snail host, and its definitive hosts. The numbers of **F. hepatica** present are usually not great enough to destroy a high percentage of the definitive hosts. There is always a favorable habitat for the snail host, and there is always a supply of herbivorous mammals at hand to act as definitive hosts.

SUMMARY

1. Up the present time **L. truncatula** is the only one of the known snail hosts of **F. hepatica** found in Turkey.
2. These snails occur in many, if not all, areas of the Anatolian Plateau.
3. Specimens naturally infected with parthenitae of **F. hepatica** were found.
4. Specimens of this species that were exposed to miracidia of **F. hepatica** became infected, developed rediae and cercariae, and discharged the latter.
5. Some snails of this species collected in March discharged cercariae on the ninth day after they were taken to the laboratory.
6. Specimens of **L. pereger** that were exposed to miracidia of **F. hepatica** did not develop rediae or cercariae.

ÖZET

1 — Şimdiye kadar yaptığımız araştırmalara göre Lymnaea truncatula Türkiyede Fasciola hepatica'nın yegâne arakonakcısıdır.

2 — Bu sümüklüler her yerde değilse bile Anadolu yaylasının bir çok bölgelerinde görülmektedir.

3 — Bu sümüklü türünün tabii şartlar altında Fasciola hepatica'nın muhtelif gelişme safhalarını taşıdığı da görülmüştür.

4 — Laboratuvarda F. hepatica miracidiumları ile enfekte edilen bu sümüklülerde redi, serker teşekkül etmiştir.

5 — Martta toplanan L. truncatulaların bazıları laboratuvara getirildikten 9 gün sonra serker çıkarmışlardır.

6 — F. hepatica miracidiumları ile enfekte edilmeye çalışılan Limnaea pereger, Succinea nevi ve Gyraulus hebraicus, larda redi ve serkeri teşekkül etmemiştir.

7 — Bulduğumuz diğer sümüklü türleri üzerinde araştırmalarımız devam etmektedir.

REFERENCES

Güralp, N., 1957 — Memleketimizde gevişenlerde Distomatose ve tedavisi. Güzel İstanbul Matbaası, Ankara.

Kendall, S. B., 1949 — Bionomics of **Limnaea truncatula** and the Parthenitae of **Fasciola hepatica** under drought conditions. J. Helminth. **23**, 57-68.

———————— **1959.** Snail Hosts of **Fasciola hepatica** in Britain. J. Helminth. **24**, 63-74.

———————— **1953.** The Life - History of **Limnaea truncatula** under Laborator conditions. J. Helminth, **27**, 17-28.

Oytun, H. Ş., 1953 — Genel Parazitoloji ve Helmintoloji, 2 inci tabı, Ankara Üniversitesi Basımevi, Ankara.

Shaw. J. N., and Simms, B. T., 1930 — Studies in Fascioliasis in Oregan Sheep and Goats. Oregon Agr. Bul. 266, Corvallis, Oregon.

Thomas, A. P., 1883 — The Life History of the Liver Fluke **(Fasciola Hepatica).** Quart. Jour. Micro. Sci., **23** (n.s.) 99 - 133.

Tüzdil, N., 1936 — Mezbahalara mahsus Parazitoloji ve tatbikatı, Ahmet İhsan Basımevi, İstanbul.