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BIONOMICS OF FASCIOLA GIGANTICA IN TURKEY

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Fasciola gigantica is one of the destructive internal parasites of ruminants in Turkey. Oytun (1942) was the first to identify it in this country. It had probably infected Turkey's livestock for many years before this, as livestock owners already knew two types of flukes occurred in their domestic ruminants. In some parts they called **F. gigantica** «snake fluke» and **F. hepatica** «leech fluke» or «leaf fluke».

F. gigantica has been reported from many areas in Turkey, often from the western and southern parts of the country and less frequently from the Anatolian Plateau. Since it is quite similar to **F. hepatica** in appearance it is quite possible that each has been mistaken for the other. It is impossible, then, to be sure that it occurs in every section from which it has been reported or that it is not present in areas from which it has not been reported. Furthermore the rather general practise of moving livestock from one section of the country to another may result in its being found in places where it is not actually established.

Both veterinarians and livestock owners believe it is invading new territory. They say it has appeared for the first time in recent years in some areas soon after rice production under irrigation was started.

It is not as widespread as **F. hepatica** but both veterinarians and owners who have had experience with it think it is more destructive. They report it will kill cattle and buffalo as well as sheep and goats.

This paper reports the results of studies of this fluke in Turkey. Such studies included some observations on its occurrence and distribution, collection, identification, and hatching of its ova, collection of possible snail hosts and maintaining them; rearing snails in the laboratory, exposing snails to **F. gigantica** miracidia; identifying **F. gigantica** cercariae which emerged from exposed snails and exposing susceptible animals to, and infecting them with such cercariae.

Materials and Methods

1. **Distribution of *F. gigantica* in Turkey.** Data on distribution and occurrence of this parasite were obtained in connection with collection of ova for hatching. The gall bladders and bile ducts of recently slaughtered cattle, sheep, and buffalo were examined both in abattoirs and on farms. The liver tissue was not examined for immature flukes.

2. **Collecting and hatching ova.** Ova were obtained from both gall bladder bile from infected animals and from mature flukes. The usual procedure of collecting bile from gall bladders of infected animals, diluting it with two or more parts water, setting the containers aside for 5-10 minutes or until the ova settled, decanting most of the liquid, and repeating the dilution and decanting until no bile color could be detected, was followed. Ova collected from gall bladders were usually mixed with those of *F. hepatica* because nearly every liver in which giant flukes were found contained the common liver fluke also. If both species of flukes were present, some of the ova were measured to determine whether or not there were significant numbers of *F. gigantica* ova in the suspension.

Ova were collected from mature flukes by cutting away with scissors most of the tissues surrounding the uterus, tearing out the uterus, teasing it open, and separating the ova from it by agitation. Larger masses of extraneous tissue were removed from the suspension with dissecting forceps. All these manipulations were in tap water. The ova were washed several times in tap water before they were stored. Measurements were made of ova from several individual flukes. These were held separate from other ova.

Ova collected by either of these procedures were held in either petri dishes or covered glass jars. In most instances they were kept on a laboratory table in light but not exposed to the direct rays of the sun. A few of the ova-containing jars were stored in a refrigerator at about 4°C. for two to four weeks and then transferred to the table. Ova were washed by decanting at intervals of one to five days during the period they were held for hatching.

3. **Collecting and maintaining possible snail hosts.** Fresh-water snails were collected in both western and southern Turkey in or near areas in which *F. gigantica* has been found and in many places on the Anatolian Plateau. Specimens of each species found were taken to the laboratory at the Veterinary Faculty of Ankara University. There they were kept in glass containers; usually either half-liter size yogurt bowls (about 7 cm high and 10 cm in diameter) or petri dishes. In most instances not more than 10 to 12 snails were placed in a single container. They were fed dead grass, fresh lettuce, oatmeal, and occasionally dead leaves from deciduous trees. Many containers were left either uncovered or only partially covered but if snails

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had a tendency to crawl out of the containers covers were used. Such covers were never air tight.

Water in the containers was changed irregularly; sometimes on alternate days, at others every third to seventh day depending upon the amount of excrement that accumulated. Containers were washed if they became badly soiled. They were kept on a table in the laboratory, all exposed to daylight but most of them not to direct sunlight. Temperatures varied from about 16°C. to 28°C. but were 18 to 24°C. most of the time. Daily fluctuations were usually not more than 2°C.

4. Hatching and rearing snails. Many of the snails produced ova in the laboratory. In most instances the ova clusters were attached to either the wall or the bottom of the glass container but a few were on leaves of grass or bits of lettuce. In some cases they were left where they were deposited and in others they were detached and put in a petri dish. They were held on a laboratory table at room temperature until they hatched. Young snails were given the same feed that was given the older ones.

5. Exposing snails to miracidia. Snails were exposed to active, recently hatched miracidia in either stendor or petri dishes. In most instances these miracidia were from gall bladder fluke ova. Both *F. gigantea* and *F. hepatica* miracidia were probably present in the cultures used for most exposures. If miracidia were numerous in the cultures some of them were poured off without removing many ova with them. If only a few miracidia were present the snails were placed in the container with them and the ova. In most instances the snails were observed with a stereoscopic microscope during exposure. They were taken out of the dish as soon as two or more miracidia appeared to be firmly attached. In some instances the miracidia attacked so quickly that a dozen or more were attached before a snail was removed. In other instances, and specially when the miracidia were scarce, none were seen attached. The number of swimming miracidia in the container seemed to decrease in some few exposures in which none were seen attached. After exposure snails were held in yogurt bowls or petri dishes and fed the usual ration.

6. Identification of cercariae. Emerging cercariae were studied in order to develop means of identifying them and specially of differentiating them from *F. hepatica* cercariae.

7. Exposing and infecting susceptible animals. Rabbits and lambs were used. The rabbits were raised in captivity. The lambs, all males, were bought on the open market. Before exposure the feces of each animal were examined for fluke eggs; all were negative.

Metacercariae given to rabbits were loosened from the glass wall of container, picked up with a medicine dropper, and put either on the base of the tongue or in the anterior pharynx. Water was given with the dropper immediately afterward. In most instances 25 metacercariae were given each rabbit. Most of them were given at from the first to the tenth day after encystment.

Each lamb received 300 metacercariae none of which were more than 35 days old. All of them had been either under water or at water's edge since they encysted.

Some were on small bits of lettuce that had been put in the containers. Others were on walls or bottoms of containers. Lettuce with metacercariae attached was put in gelatine capsules and these were introduced into the pharynges with forceps. Metacercariae on walls or bottoms were loosened, picked up with a medicine dropper, and dropped on a small piece of paper tissue. The tissue was given in a gelatin capsule.

RESULTS.

1. *F. gigantea* were found in cattle livers at the Adana and Bursa abattoirs, in both cattle and sheep livers in all seasons of the year at the Ankara abattoir, and in livers of sheep sacrificed in two villages near Lake Apolyont. The local veterinary inspectors said the infected cattle found at Adana and Bursa were raised locally; the origins of the infected cattle and sheep seen at Ankara could not be determined; and the infected sheep sacrificed in the villages were, according to their owners, born and raised in the immediate vicinities. Flukes were not found in lambs or calves under eight months old. These parasites were collected from bile ducts only; never from gall bladders.

2. *F. gigantea* ova held in the laboratory hatched in from 16 to more than 40 days. Those hatching in the shortest time were held at temperatures of 20 - 23°C. At lower temperatures it took longer. But this was not the only factor as the hatching time of ova kept in the same container varied from 20 to more than 40 days. Ova from gall bladders hatched in higher percentages than did those teased from flukes.

Sizes of ova measured were, in most instances, somewhat similar to those reported by Kendall and Parfitt (1959) and others. The average for 33 ova was 158 microns by 88.4. Measurements of a few ova teased from each of several flukes indicated there is some difference in sizes depending upon the fluke of origin. The few ova from the gall bladder of a rabbit that were measured were narrower than are those from sheep and cattle.

3. Species of lymnaeid snails collected in Turkey, their origins, and seasons in which collections were made, were as follows: (Table I).

Table I

Species (*)	Collected at	Date
<i>Lymnaea truncatula</i>	Near Mediterranean, many places near Marmara Sea, many places on Anatolian Plateau.	All seasons
<i>L. peregra</i>	Many places on Anatolian Plateau, near Kozan in South Turkey	All seasons
<i>L. palustris</i>	Lake Apolyont; near Bursa	March, July
<i>L. auricularia</i>	Near Kozan (Alapınar) and rice irrigation ditches around Kozan in South Turkey, near Dörtıyol in South Turkey and Seyhan River. Lake Apolyont in West Turkey; Çubuk or Black Lake (El. 1500 m.), near Kızılcahamam (El. 1000 m.), and near Malatya, all on Anatolian plateau.	September, October, December July July

L. auricularia was found in clear water lakes containing much vegetation, in permanent running water, in rice irrigation ditches some of which become dry during the fall months, in a pond with a mud bottom, and in a small, clear, sluggish, shallow stream. They were never found in water devoid of vegetation. They were always under water; on plants, rocks, or the mud or silt bottom.

The color of different specimens of this species varied considerably. Those from near Dörtıyol were a very dark brown but those from Lake Apolyont, Çubuk Lake, and a stream near Kızılcahamam were yellowish - brown. The shells, regardless of origin, were thin, translucent, and almost white. Shapes varied considerably the width being from 53 to 57 % of the length.

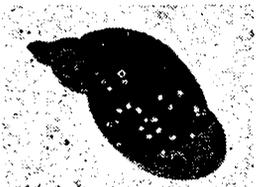
4. *L. auricularia* reproduced well under laboratory conditions. They deposited ova in clusters of from three or four to above 50 at all seasons of the year. These hatched satisfactorily in from 12 to 25 days if kept in water at room temperature. The young ate lettuce and dead grass fairly well and oatmeal very well. They reached lengths of five to seven mm in 60 days.

5. Newly - hatched *F. gigantica* miracidia attacked snails of all the four species of *Lymnaea* found in Turkey. They seemed to attack *L. auricularia*

(*) Dr. C. A. Wright, British Museum of Natural History, London, identified all these except the specimens collected at a point near Malatya which were tentatively identified by the Authors. Dr. Wright identified many other specimens also. His willing and competent assistance is gratefully acknowledged.

most vigorously; *L. peregra* appeared to be second most attractive to them. They were observed penetrating the soft parts of these two species but not of the other two. Specimens of each species were crushed and examined 25-35 days after exposure. Parthenitae were found in *L. auricularia* only.

6. **Cercariae** emerged from exposed *L. auricularia* but not from the other species. Emergence was observed 46 to 60 days following exposure. No snail, known to be infected, ever became free of parthenitae. These cercariae were quite similar to those of *F. hepatica* but they differed in the following respects: they did not swim as rapidly; they had a greater tendency to swim in circles, usually counter clockwise; they swam for a longer time before encysting; some of them encysted on the shells of their vectors (Fig. 1) while this was never observed with *F. hepatica* cercariae; they had a greater tendency to encyst on the bottoms rather than the sides of the glass containers; they had a greater tendency to encyst on lettuce or other plant material; the tail remained attached to the body much longer during encystment, with few exceptions the tail was motionless or nearly so when it became detached from the encysting body (the tail of *F. hepatica* may swim vigorously after it becomes detached); and the excysted metacercariae are larger (The average greatest diameter of 44 excysted *F. gigantica* that were measured was 193 microns that of 68 *F. hepatica* 174 microns.).



(Fig. 1) *Lymnaea auricularia* with *F. gigantica* metacercariae attached to its shell. X3.

7. Rabbits and a single lamb developed flukes in their livers after they were given metacercariae from *L. auricularia* that had been exposed to *F. gigantica* miracidia. The prepatent period in the rabbit was 90 days. The following is a protocol of the lamb.

Lamb No. 332 acquired in July, 1960

10/8/1960 Weight 16.5 kilos

20/8/1960 Given 300 metacercariae, 200 not more than 7 days old and encysted on lettuce, 100 from walls and bottom of glass container and transferred to paper tissue for administration.

22/9/1960 Weight 17 kilos: has shown no symptoms of fascioliasis.

6/10/1960 Weight 20 kilos: Slaughtered. Small amount of clear liquid that did not clot on exposure to air in peritoneal cavity; surface of liver slightly roughened but with no tags; circumscribed discolored areas .2-.5 cm in diameter on surface of liver; hemorrhages in liver tissue; flukes up to 4X1.5 mm in liver tissue; no flukes found in either bile ducts or gall bladder.

Discussion

The definite identification of specimens of *L. auricularia* collected on the Anatolian Plateau as well as in the warmer areas of Turkey gives credence to reports that *F. gigantea* is widely distributed in the country. It seems probable that low temperatures do not protect Turkey from this snail as specimens were collected at an elevation of 1,500 meters. It is probable that *F. gigantea* will become an increasingly serious parasite unless methods of control developed and used. The movement of susceptible animals from infected areas to far - removed grazing grounds, which is a common and economical practice, facilitates the spread of this and other parasites. Increased emphasis on irrigation farming is resulting in more irrigation ditches; and these are, in at least some instances, favorable sites for the snail host.

S u m m a r y

1. *F. gigantea* is rather widespread in Turkey.
2. *L. auricularia* has been found to be a snail host in this country. Attempts to infect other species of *Lymnaea* failed.
3. This snail occurs in Western and Southern Turkey and on the Anatolia Plateau up to 1,500 meters elevation.

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Ö z e t

1. *Fasciola gigantea*'nin Türkiye'de yayılışı *F. hepatica* kadar geniş değildir.

2. *F. gigantea*'nin biyolojisi üzerinde yaptığımız araştırmalarda *Lymnaea auricularia*'nın, bu parazitin Türkiye'de yegâne arakonakcısı olduğunu tesbit ettik. Bu sümüklüye, *F. gigantea*'nin bulunduğu evvelce bildirilen bütün bölgelerde ve şimdiye kadar mevcut olduğu tesbit edilmeyen bazı mıntakalarda da rastladık.

3. *L. auricularia* Adana da Kozan ilçesine bağlı Alapınar köyünün Bekirce mevkiinde, Kozan ilçesi etrafındaki piriñ tarlalarının sulama kanallarında, Dörtüyolda, Seyhan nehrinde bulunduğu gibi, Bursa civarındaki Apolyont gölünde de fazla miktarda görülmüştür.

Aynı sümüklüye Çubuk civarındaki Karagölde (Yükseklik 1500 metre), Kızılcahamamda (Yükseklik 1000 metre) ve Malatyada da rastlanmıştır.

L. auricularia'nın deniz seviyesinden 1000 - 1500 metre yükseklikteki mevkilerde de bulunabilmesi enteresandır. Elimizde mevcut literatür bu sü-

müklünün daha çok subtropik iklime malik sahalara yayıldığını bildirmektedir.

L. auricularia'nın Anadolu yaylasında da görülmesi bize *F. gigantica*'nın her an bu bölgeye de yayılabileceği endişesini vermektedir.

4. Lâboratuvârda *F. gigantica* miracidiumları ile enfekte edilen *L. auricularia*'lar, 46 - 60 gün sonra serker çıkarmaya başlamışlardır.

5. 300 metaserker verdiğimiz bir kuzu enfektiondan 57 gün sonra kesilmiş, karaciğerinde 4X1.5 mm. büyüklüğünde ve henüz tenasüli olgunluğa erişmemiş *F. gigantica*'lara rastlanmıştır.

25 metaserker verdiğimiz tavşanların 90 ıncı gündeki gaita muayenelerinde *F. gigantica* yumurtalarına ve aynı günkü otopsislerinde de safra kanallarında olgun parazitlere rastlanmıştır.

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