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STUDIES ON THE BIOLOGY OF PARAMPHISTOMUM CERVI SCHRANK, 1790 IN SHEEP IN THE DISTRICT OF ESKİŞEHİR ÇİFTELER STATE FARM

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Eskişehir Çifteler Harası Yöresinde Koyunlarda Paramphistomum cervi Schrank, 1790'nın Biyolojisi Üzerinde Çalışmalar

Özet: *Çifteler Harası yöresindeki otlaklardan* 4068 *Planorbis planorbis,* 2041 *Valvata macrostoma,* 1471 *Aplexa hypnorum,* 167 *Lymnaea truncatula,* 17 *Planorbis carinatus,* 6 *Lymnaea auricularia ve* 1 *Succinea pfeifferi toplanmış ve P. cervi doğal enfeksiyonu yönünden bunların bakıları yapılmıştır.*

Yalnızca 4068 *P. planorbis'ten* 64*'ünde (%* 1.57*) P.cervi gelişim dönemlerine rastlanmıştır. Nisan ve kasım ayları arasında her ay enfekte P.planorbis'lere rastlanmış, maksimum enfeksiyon ekim ayında (%* 2.20*) bulunmuştur.*

P.cervi yumurtaları Çifteler Harası mezbahasında kesilen koyunlardan sağlanmıştır. Laboratuvarda S.pfeifferi dışında 6 *çeşit sümüklüböcek, yumurtalardan gelişen P.cervi miracidium'ları ile enfekte edilmiştir. Yapay enfeksiyonlarda da yalnızca P.planorbis'ler duyarlı bulunmuş, diğer sümüklüböceklerde hiçbir gelişme olmamıştır. Sümüklüböceklerde enfeksiyon oranının, miracidium sayısına ve sümüklüböceklerin büyüklüğüne bağlı olduğu görülmüştür. P.planorbis'lerdeki P.cervi gelişim dönemleri incelenmiştir.*

Onbeş kuzu 1000 *er P.cervi metaserkeri ile enfekte edilmiştir. Bu hayvanlarda enfeksiyon oranı %* 19.6-77.0*, prepatent süre* 102-142 *gün olarak bulunmuştur.*

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Summary: *From the infected farm pastures* 4068 *Planorbis planorbis,* 2041 *Valvata macrostoma,* 1471 *Aplexa hypnorum,* 167 *Lymnaea truncatula,* 17 *Planorbis carinatus,* 6 *Lymnaea auricularia and* 1 *Succinea pfeifferi were collected and controlled for the natural P.cervi infection.*

Only 64 *out of* 4068 *P.planorbis* (1.57 %) *were found infected with the larval stages of P.cervi. From April to November* 1977, *natural infected P. planorbis were found every month with the maximum infection in October* (2.20 %).

P.cervi eggs were obtained from sheep at necropsy, in the Çifteler State Farm. Six different snail species with the exception of S.pfeifferi were exposed to P.cervi miracidia cultured in the laboratory.

Among the snail species only P.planorbis were found susceptible to P.cervi infection. No development has occurred in the other snails.

The infection rates were found related with the number of miracidia and the size of the snails. Different development stages of P.cervi in P.planorbis were investigated.

Fifteen lambs were infected with 1000 *P.cervi metacercariae. The infection rate in these animals was found to be between* 19.6–77.0 %. *The prepatent period was found* 102–142 *days in the infected animals.*

Introduction

There are many references to the infections caused by immature specimens of the Paramphistomidae family resulting in serious economic losses and deaths in ruminants (16).

There are still gaps in our knowledge about the incidence, distribution, treatment and particularly pathogenicity of various species of paramphistomes in Turkey. Outbreaks of paramphistomiasis among ruminants in Turkey are not common, and serious disease signs and losses due to these parasites were not recorded in both sheep and cattle. Paramphistomiasis is caused by massive infection of the small intestines with immature paramphistomes, thus eggs are not recorded in the faeces and the immature forms are not easy to detect during the autopsies. Therefore, these handicaps may prevent the portrayel of the actual situation of paramphistomiasis in ruminants. Some slaughterhouse examinations which were conducted recently revealed that the distribution of paramphistomes in our ruminants were at a rate that one should not overlook.

In Turkey, the district of Eskişehir, Çifteler State Farm is one of the areas where P.cervi is widespread in sheep. In this region, the occurrence rate of these parasites in sheep is recorded between 57.7–100 % (15).

The life cycles of paramphistomes resemble one another in each species, and one kind of snail species play the role of intermediate host, same as in most trematode biology.

However, these snail species show a variation according to the species of the paramphistomum and according to the geographical regions of the world (16, 17, 24, 25).

Bulinus syngenes, B.alluaudi in Kenya, B.truncatus in Iraq and Egypt, Planorbis planorbis in Bulgaria, Indoplanorbis exustus in India, P.planorbis, B. contortus and B.truncatus in Italy, P.planorbis and Anisus vortex in Germany were noted as intermediate hosts for P.cervi (1, 3, 5, 6, 9, 10, 18). New analysis of the intermediate host spectrum of paramphistomes suggest that parasites whose intermediate hosts are Bulinid snails were not P.cervi (27, 28).

The purpose of this study is to shed some light on the biology of P.cervi.

Materials and Methods

Our studies were carried out both in the field and in the laboratory.

Between April and November of 1977, every month, at least one time, this farm pasture and surrounding area were visited and controlled.

The presence and types of freshwater snails in the area were determined and samples were obtained and also seasonal changes were recorded. These snail specimens were identified according to the shell morphology by Dr. N.J.Evans (14) from the British Museum. During the same period, in the Farm's slaughterhouse, the amounts of infected sheep with P.cervi were determined and the sample of P.cervi eggs which were necessary for our laboratory studies, were obtained from mature parasites present in these infected sheep. The identification of these mature paramphistomes were made by Dr. O.Sey (26) from Hungary in accordance with histological sections.

In the laboratory, snails were controlled under the steromicroscope for determination of natural infection. Naturally infected snails were separated and the others were held one month in the laboratory and at the end of this period, were controlled again in the same manner and the final natural infection situation of snails were noted. Species of snails which were naturally infected and their numbers were recorded and the infection rates were determined monthly.

Mature worms which were collected from the infected slaughtered sheep in the Farm's slaughterhouse were transferred into the laboratory in the saline solution (0.9 %) on the same day. These viable mature worms were placed in the incubator (37°C.) nightly and the next day eggs containing water were separated from the parasites by means of a mesh and were washed 2–3 times by distilled water.

In the laboratory, factors influencing the development of P.cervi eggs such as temperature, light and variable mediums (distilled water, spring water, tap water = chlorinated water and physiological saline solution) were investigated. Fully developed egg cultures were placed under light source in a 30 cm. distance to secure the miracidial hatching. The movement and survival time of miracidia were investigated.

In the laboratory six different snail species of various sizes with the exception of S.pfeifferi were infected by newly hatched P.cervi miracidia in various numbers. In the laboratory–infection of snails, 5, 10 and 15 miracidia were used.These miracidia were taken one by one by means of a pipet and placed into the haemagglutination plate holes and soon one snail was put into each hole. Thus snails were kept together with the desired number of miracidia for eight hours. In these infection experiments, the efficacy of snail size, number of miracidia used and the infection time were investigated and the infection rate was determined.

In the infected snails, earliest detection of different development stages such as a sporocyst, redia, and cercaria were recorded and their location in the snail tissue were noted.

In the snails, these developing stages were revealed by means of snail dissections without using any stain. Only half suspensed India ink was used to cause the cercarial mucoid–fin to be visible and chloroform was used to secure cercarial inactivity.

Cercarial shedding of infected snails in the boxes were prevented by means of a black cloth cover. When shedding of new cercariae

were needed, snails were exposed to light. Thus, snails were placed into the petri dish lined with a plastic sheet and put under the light source (75 watt) with a 30 cm. distance and encystment was secured on the plastic sheet. Encystment process was observed and influencing factors were recorded. Metacercariae on the plastic sheet were kept among the wet filter paper in the refrigerator.

Infestation experiments on sheep were conducted on 15 Karaman lambs which were 5–6 months old. Faecal samples from each lamb were checked and none were found infected. Three out of 15 lambs were infected with metacercariae which were obtained from the naturally infected P.planorbis and, the others were infected with metacercariae from the experimentally infected P.planorbis. Each lamb received 1000 P.cervi metacercariae that were 3 to 20 days old and which were viable.

The presence of Paramphistomum eggs in the sheep faeces were established by petri–sedimentation technique. After the first detection of P.cervi eggs in the faeces of infected animals, autopsies were performed on these sheep within one week.

At the autopsies, rumen, reticulum, omasus and anterior parts of the small intestines were controlled and the collected parasites were counted and the infection rate was determined by comparing the number of metacercariae given into these animals.

Results

A– Field Studies

From the area pastures (Fig. 1, 2) 4068 Planorbis planorbis, 2041 Valvata macrostoma, 1471 Aplexa hypnorum, 167 Lymnaea truncatula, 17 Planorbis carinatus, 6 Lymnaea auricularia, 1 Succinea pfeifferi snails were collected and were checked for the natural P.cervi infection. Among these snails natural P.cervi infection was detected only in P.planorbis (Fig. 3, 4). Sixtyfour out of 4068 (1.57 %) P. planorbis was found to be naturally infected between April and November of 1977. Infected P.planorbis were detected each and every month; infection was found to be least prevalent in April (0.75 %), and more prevalent in October (2.20 %). Infection was determined in the middle and big size P.planorbis between April to August. In the small size P.planorbis, along with the big and middle size P.planorbis, infections were determined after the beginning of September.

While the infection of P.planorbis was 1.57 %, P.cervi occurence rate found among slaughtered sheep, during this same time span, was 80.76 %.

B- Laboratory Studies

1- *Characteristics of P.cervi Eggs:* P.cervi eggs (Oval, operculated, smooth-surfaced and white-greyish in colour) that were obtained from the mature parasites of infected sheep, were measured 121-169 microns in length and 68-95 microns in width. At the posterior pole of the eggs, a small button-like projection was generally asymmetrically situated (Fig. 5).

In the centre of the egg, embryo was seen in an early segmentation stage (which commonly consisted of 4 blastomers) and surrounded by yolk cells.

At the operculated anterior pole, egg and operculum connection secured by means of teeth that were situated at the rims were detected and this structure became visible in the miracidium-developed or hatched eggs (Fig. 6).

2- *Factors Influencing the Development of P.cervi Eggs:* In the laboratory, factors influencing the development of P.cervi eggs such as temperature, light and variable water mediums were investigated and temperature was found to be the most effective factor.

The development of P.cervi eggs in variable water mediums did not make any difference. In variable water mediums having the same temperatures, hatching of miracidia occured on the same periods.

A very small percentage of eggs developed at 17°C. The development process did not occur all at the same time. Therefore miracidia began to hatch on the 27 th day and this process continued nearly one month without being in a mass manner.

Eggs hatched on the 13 th day at 26-27°C., and on the 12 th day at 29°C. At these temperatures, a mass-manner hatching of miracidia occured one or two days after their first detection and continued as long as one week in a decreasing frequency.

At the temperatures of 32°C. and 35°C., one or two miracidia were seen on the 9 th day but a mass-manner hatching of eggs occurred on the 11 th and 10 th days respectively and continued for 3-4

days. At these temperatures, higher than 30°C. an appreciable percentage of eggs failed to hatch. At 42°C., P.cervi eggs did not develop. Light does not seem to have any effect on the development of the miracidium. In the eggs incubated in the dark or light, miracidia developed at the same period, if the temperatures were equal.

P.cervi eggs were preserved one month in the refrigerator (at 4°C.) without any development, and miracidia hatched on the 11 th day after they were placed in the incubator at 29°C.

Miracidia did not hatch from the egg cultures which their water freezed or dried totally. When the miracidium-developed eggs were placed into the refrigerator to prevent early hatching (For obtaining miracidia on time), miracidia emerged late and never hatched in mass numbers after they were transferred into the laboratory conditions.

3- *Development in the P.cervi Eggs:* The transparency of the P. cervi egg shell permitted the observation of the development. At the 26–27°C., within the first 4 days no marked changes occurred in the eggs except the segmention of the embryo. On the 6 th day of the development; length of the embryo has increased and the yolk cells decreased. At this stage embryo measured an average of 47×79 microns. On the 9 th day of development the embryo measured an average of 116×63 microns and it was located along the one side of the egg. On the 11 th day of the development, yolk cells wholly disappeared and the developed miracidium was seen in a limited activity. At this stage opercular line became visible in the egg. On the 13 th day, miracidium measured an average of 185×65 microns and, filled the egg by its entire length. The miracidium was very active and from time to time its anterior part pressed the operculum.

Activity of miracidium successively increased till the hatching and eggs' operculum either completely fell away from the rest of the shell or pushed outwards in a door-like fashion (Fig. 6).

4- *Factors Influencing the Hatching of Miracidiae:* At first, the anterior part and soon other parts of the miracidium were introduced through the opercular opening and emerged by their rapid body extentions and contractions. At this phase, the cilial movements were seen more vigorously especially on the part of the body still within the egg.

Light was found to be more effective factor for influencing the hatching. When the completely-miracidium-developed eggs' culture

were placed under the light source, miracidia emerged after 15–20 minutes of exposure to light. With contrast to this, when the eggs that were in the same stage were kept in total darkness, hatching procedure was delayed markedly.

In the egg cultures on different temperatures hatching did not occur on the same day nor did they terminate on the same day. For example, some eggs hatched on 13 th day at 26–27°C., others hatched as late as one week.

P.cervi miracidium was in the shape of a torpido, covered with cilia and carried no eyespots (Fig. 7). Their size were measured to be 215—253×49—65 microns.

Newly hatched miracidia swam more actively in the distilled water, spring water compared to tap water and principally in physiological saline solution.

At the 26–27°C. temperatures, all miracidia survived for 4 and 8 hours. Sixteen hours after hatching, only 1/10 of the miracidia were found to survive but they had lost their activity.

5– *Factors Influencing the Infection of the Snails:* In the laboratory, six different snail species of various sizes with the exception of S.pfeifferi were infected by P. cervi miracidia in various numbers and, only in the case of P.planorbis, development stages were detected.

Infection rate among small and middle sized P.planorbis is higher while their mortality rate was found higher too.

Infection rate of P.planorbis which were infected with 5, 10 and 15 P.cervi miracidia was found to be 37.22, 53.37, 57.69 % respectively. It was also observed that the infection rate increased with the number of miracidium used. But, when the snails placed into the egg culture which contained large proportions of miracidia, snails' reaction and excretion were increased. Thus, it was also observed that too many miracidia had a negative effect on the P.planorbis infections.

At the infection of P.planorbis with 5 miracidia performed in August, infection rate was found to be 37 % and in November 34 %. Thus, no important seasonal variations were recorded in the infection rate. However, the development of larval stages in the snails (From, sporocyst to emerging cercariae) was slower in November as compared to August, when they were shedding cercariae as of the 35 th day of infection. Since cercariae shedding of P.planorbis infected in Novem-

ber were markedly delayed and the dark pigmented cercariae of P. cervi were detected in the snail tissue still on the 74 th day of infection.

6– *Penetration of the Snails by Miracidia and the Development of Larval Stages:* The transparency of the shell and mantle tissue in the young snails facilitated the observation of miracidial penetration.

Most miracidia become quite agitated when they are in the vicinity of the snail. However, some exhibit no attraction to the snail and some are attracted to the snail faeces for a long time. Agitated miracidiae swim a short eliptical course around the snails and they do not leave the head, foot and tentacle of the snails alone. Eventually some miracidia entered from the mantle cavity and soon swam out of the snail again.

It was seen that miracidium penetrated the P.planorbis only through the mantle cavity and not from the head, foot or tentaculum.

The miracidium within the mantle cavity apply its anterior part to the posterior mantle wall and usually within 5 minutes the anterior part is completely embedded in the snail tissue. At this stage, the snail reacts vigorously and this snail reaction sometimes was found to be sufficient to discharge the miracidium out of the snail. The complete process of penetration lasted about 15–20 minutes or more.

a) *Sporocyst:* Development of larval stages in the snail was observed by the dissection of snails. Location and identification of sporocysts, especially in the early stages of development were difficult to detect, but this difficulty was reduced in the young snails due to their scant amount of snail tissue.

At the beginning of penetration, no morphological changes were observed in the miracidium and the attaching miracidium could become free and could swim out again but unbalanced and slow in manner.

The miracidium embedded its anterior part in the snail tissue, was covered completely with cilia and its body showed marked swelling differentiations (Fig. 8).

The transformation of miracidium into sporocyst was not immediate. One to 2 hours after the infection of the snail by the miracidium, it was observed that the miracidium was shorter and its body was divided into two parts with a constriction and its cilia at the anterior part still persisted (Fig. 9).

Earliest detection of sporocysts, sacculer in shape, was two days after the infection (Fig. 10).

Eight days after the infection the sporocysts were elongated or curved in shape and the developing rediae in it were observed (Fig. 11).

Earliest detection of mature sporocyst in the P.planorbis was twelve days after the infection. In this sporocyst 11 rediae were counted whose pharynx and intestines could be identified and was measured to be 1900×810 microns. (Fig. 12). Sporocysts were detected mainly around the intestines and in the mantle tissue.

b) *Rediae:* Earliest detection of rediae, free of sporocysts, were 12–13 days after the infection. These young rediae were opaque, slightly curved in shape and had a limited activity (Fig. 13). In the young rediae the pharynx and the intestines were visible and they contained embryo balls from which cercariae or daughter rediae would develop (Fig. 14). It was observed that a week was necessary for the birth pore to become visible. Rediae were detected around the intestines, hepato-pancreas and in the mantle tissue of the P. planorbis.

The developing rediae showed great variations in size and the mature rediae measured 700–1150 microns in lenght and 125–250 microns in width.

c) *Daughter rediae:* The daughter rediae were observed in P. planorbis in different months of the year. The production of daughter rediae were less compared to the regular production of cercariae. Daughter rediae were observed after 40 days of the infection. It was observed that, the localization in the snail tissue was an important factor rather than the seasonal factors for the production of daughter rediae. Mostly daughter rediae were developed from the rediae which were situated at the lateral part of the mantle tissue of the snail and they were easily identified at the first external circle of the snail before its dissection.

Developing daughter rediae were distinguished from the developing cercariae by their pharynx, their whitish-grey colour and their longitudinal arrangement in the body of the rediae (Fig. 15, 16).

d) *Factors Influencing the Emergency of Cercariae:* Early free immature cercariae were detected in the snail tissue after 24 days of the in-

fection. Thus, immature cercariae were liberated from rediae before their complete development. Like the rediae the developing cercariae show great variability in size at the beginning. In the young cercariae, the body was markedly small, eye spots were prominent and pigment was only accumulated around them. The tail was short and wide rather than long (Fig. 17).

Within the next few days, the immature cercariae gradually increased in size and the tail became longer. The eye spots were very prominent and dark. The pigment around them were like irreguler blotches and soon pigmentation covered all the body in rows and branches (Fig. 18).

The mature cercariae were large, quite active and dark pigmented so, they were easily recognized before the P.planorbis dissection (Fig. 19). Their body was 300–480 microns long and 180–325 microns wide while the tail was 425–510 microns long and 75–80 microns wide.

The mature cercaria is a vigorously swimming organism and its body made relaxation and contraction movements. It has a pear–shaped body, and its posterior part is wider than its anterior (Fig. 20).

The tail is cylindrical and tapers gradually towards the posterior end. When the cercariae were placed into half diluted India ink suspension, the mucoid–fin, which is on and around the tail, (Fig. 21) became visible. In the mature cercariae the mucoid fin were seen constant in shape but in the immature cercariae, this structure was not recorded especially before emergency. Cercariae swimming direction is mostly circular but they may also swim straight or in zig–zag lines. They rise gradually to the water surface and gradually descend again and cover a long distance in a very short time.

It was seen that light was a great factor in shedding cercariae. Infected snails kept in the dark started shedding cercariae again after being exposed to light.

Under natural day light, snails continued to shed many hours but in small numbers. Under strong illumination, large numbers of cercariae emerged during the second or third hour after exposure of the snails to the light. Snails that had released cercariae under strong illumination, required a rest period at least for about 2 or 3 days.

During the experiments, cercariae were collected from the snails only one day in a week in order to regulate metacercarial age conformity and to obtain them in high numbers.

Some infected P.planorbis continued to shed cercariae in the laboratory more than 13 months and some snails were free of the infection after 11 months.

7- *Factors Influencing on the Encystment of Cercariae:* Shortly after releasing most cercariae commence encystation and become metacercariae (Fig. 22). Under the light source and, in the presence of vegetation in the water most cercariae encyst on the vegetation within 15–20 minutes.

Cercariae leaving the snails formed into metacercariae–mostly on the sides of a petri dish and rarely at the bottom–lined with a plastic sheet–within 25–30 minutes. Thus, it was seen that the encystation was slightly delayed in the absence of vegetation. Most metacercariae were seen near the surface of the water on the plastic sheet and especially on its curved parts.

When the green leaves were placed between plastic sheet and petri dish, cercariae encysted on the same parts as explained above, thus they had no effect on the encystation.

When the petri dishes were placed under the light at a distance less than 30 cm., most of the metacercariae were not encysted due to the increasing temperature of water in the petri dish.

In our studies during the collection of metacercariae, some metacercariae were ingested by P. planorbis. But these were discharged by snail faecal material and on their examination, they were found viable.

During the encystment cercaria attaches with its ventral surface, and the material for cystation begin to secrete from the pores all over the body (Fig. 23), while the tail movement increases. Tail breaks off the body of the cercaria–at the the earliest–within 3–5 minutes, but free tail motion continues for several hours. By the end of 20 minutes, a cyst formes around the cercaria. At first, the cyst wall was soft and fragile, but become hard after 2–3 days.

Metacercaria was in the form of a halfsphere and was surrounded with a thick layer of a cyst wall. These metacercariae were measured 210–255 microns in diameter (Fig. 24).

Newly formed metacercariae lost most of their activity 2 weeks after encystation but still some of their activity can be observed a very long time on their careful examination under the strong light mic-

roscope. Also eye-spots of metacercariae become invisible 2 weeks after encystation.

One fifth of the metacercariae kept among wet filter paper in the refrigerator kept their viability for about six months. Deaths started after the second month. In the dead metacercariae, embryo was seen to shrivel up and turn gray but the cyst wall persisted unchanged.

8- *Experimental Infection of Lambs with P.cervi Metacercariae:* Fifteen lambs were each infected with 3-20 days old 1000 P.cervi metacercariae. No clinical symptoms were observed after the administration of such a dose.

Prepatent period was found to be 102-142 days in the infected lambs and at the end of these periods autopsies were performed within one week. Parasites were detected in the rumen, less frequently in the reticulum and in the omasus. No parasites were detected in the abomasus and in the small intestines. A minimum of 196 and a maximum of 770 P.cervi were found from each infected animal. The infection rate was determined to be 19.6-77 % (Table I).

Parasites were kept alive 7-8 hours in a warm (37°C.) saline solution and their lenghts and widths were measured to be 3.5-5.5 mm. and 1.8-2.2mm.respectively after fixation with 5 % formalin.

Tablo I. Experimental infections of sheep

Sheep No.	The origin of metacercariae	Prepatent periods (days)	The numbers of parasites found at the autopsies				Infection rate (%)
			Rumen	Reticulum	Omasus	Total	
1	From the naturally infected P. planorbis	107	538			538	53.8
2		124	213			213	21.3
3		124	350	7		357	35.7
4	From the experimentally infected P.planorbis	102	393	5		398	39.8
5		104	192	3	1	196	19.6
6		107	742	24	4	770	77.0
7		119	282	40		322	32.2
8		121	363			363	36.3
9		124	355	11		366	36.6
10		125	376			376	37.6
11		125	210	8		218	21.8
12		125	492			492	49.2
13		127	500			500	50.0
14		141	436			436	43.6
15		142	488	7		495	49.5

Discussion

It is stated that the intermediate host of P.cervi varies according to the geographical regions. However, Planorbid type snails play the main role of an intermediate host (1, 3, 5, 6, 9, 10, 18, 27, 28). In our study P.planorbis were recorded as a intermediate host for P.cervi.

Heavy infestation of the snails with various paramphistomes are reported mainly in the late summer and autumn months and the incidence varies between 3 to 75 % in the infected areas (4, 5, 6, 9, 19, 30). In our study naturally infected P.planorbis were found each and every month between April and November; infection was found to be least prevalent in April (0.75 %) and most prevalent in October (2.20 %). Infection was determined in the middle and big size P. planorbis between April to August. In the small size P.planorbis along with the big and middle size P.planorbis, infections were determined after the beginning of September. These results have shown that the sheep could contract the infection from the infected pastures as soon as they begin to graze in the spring months. These results have also shown that the snails were infected much earlier and were able to endure the winter months while they were already infected.

Güralp (15), reported that the Çiftcler State Farm and the surrounding area is one of the areas where P.cervi is widespread in sheep in Turkey. Also we found that the occurence of P.cervi in sheep was an average of 80.76 %, contrary to this, the infection rate of P.planorbis was at an average of 1.57 %.

It was noted that the main factor for the development of P.cervi eggs was temperature rather than light (12, 21, 23). Similar results were obtained from our studies and also the development of P.cervi eggs in variable mediums did not show any differentiation provided that all the water mediums had the same temperature.

Light is an important factor which influence the hatching. P. cervi miracidia are 240 microns in size and some miracidia of Paramphistomum species kept their viability longer in lakes and pools compared with other media. Also it is note that tap water is a good medium than distilled water and saline solution (0.9 %) for some Paramphistomum miracidia (7, 21, 23). In our study light was found to be a most effective factor for hatching and it was seen that newly emerged miracidia swam more active in distilled water and spring water. Their size was measured to be 215—253×49–65 microns.

It was recorded that too many miracidia had a negative effect on the experimental infection of snails and the young (little or medium sized) snails were suitable for infection (13, 23). During the experiments it was observed that the infection rate increased with the number of miracidium used. But contrast to this, it was seen that the large proportions of miracidia had a negative effect. On the subject of snail size, it was seen that the infection rate among the small and middle sized P.planorbis is higher while their mortality rate is higher too.

Generally it was accepted that the miracidia lost their cilia during the penetration and entered the snail host tissue as a young sporocyst. On the observation of miracidial penetration into the snails, both mechanical and enzymatic effects together play a role (21, 23). The transformation of the miracidium into a sporocyst is a gradual process and no clear line can be drawn between the invading miracidium and young sporocyst (8). In our studies persistence of cilium of the anterior part of the miracidium was also observed, 1–2 hours after the infection of snails.

On the development of P.cervi in the snail host, a very limited avaible information is present, but our observation are in conformity with the papers of Kraneburg (21).

In some Paramphistomum species, the occurence of daughter rediae were recorded only in Autumn months (20). In our experiments, however, the daughter rediae were observed in P.planorbis in different months of the year. Yet, the production of daughter rediae were less compared to the regular production of cercariae and they were observed after the 40 th day of infection. In spite of the fact that there were no records on the literature, in our study also it was observed that, the localization in the snail tissue was also an important factor for the production of daughter rediae. Because the daughter rediae developed mainly from the rediae where they developed in the external circle of the snail mantle tissue. Therefore it is possible to detect the rediae which carried daughter rediae from these external lateral parts of the mantle tissue of P.planorbis before its dissection on the examination under the steromicroscope.

It has been recorded that the light was the most important factor for cercarial shedding of infected snails and the snails could continue to shed about 1–10 months (21, 23, 29). We obtained similar

results and we saw that the infected P.planorbis continued to shed cercariae in the laboratory more than 13 months and some snails were free of the infection after 11 months.

Paramphistomum cercariae can be encysted on the vegetation and other subjects in the water and they can stay viable for about 5 months when they are preserved in the refrigerator (1, 3, 21). In our studies encystation occured on the plastic sheet which was placed in the petri dish and especially around its folds. It was also observed that one fifth of the metacercariae kept among wet filter paper in the refrigerator with a temperature of 4°C. kept their viability for about 6 months, deaths started after the second month.

In the P.cervi infection prepatent period was reported to be 129 days in goats, 103–115 days in cattle and sheep and the infection rate was 45.82 % in goats, 40.6 % in sheep and 44.8 % in cattle (2,22). In our study it was determined that the prepatent period was 102–142 days and the infection rate was 19.6–77 % in lambs.

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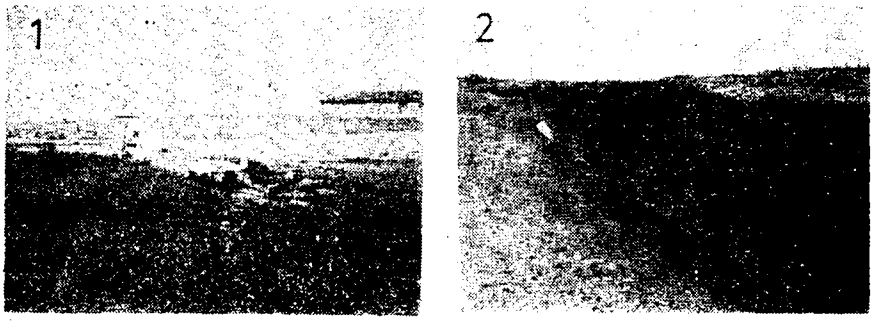


Figure 1. The trough and its continuation of wet area in Çifteler State Farm, where snails were collected
Figure 2. Canals for irrigation carrying intermediate host snails in Çifteler

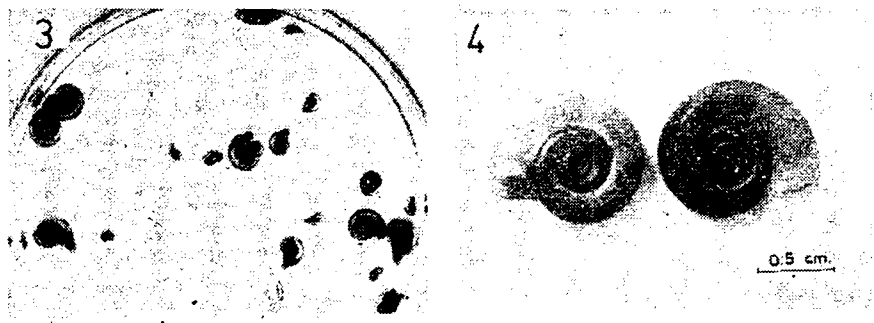


Figure 3. Planorbis planorbis, the intermediate host of P.cervi
Figure 4. The shell morphology of P.planorbis

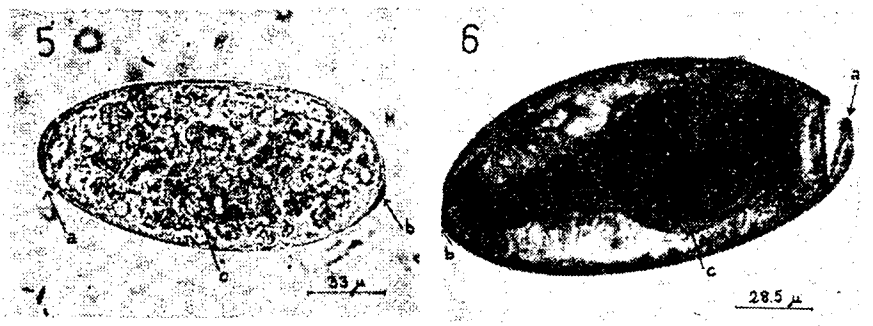


Figure 5. The P.cervi egg a) operculum b) slight projection c) embryo
Figure 6. The miracidium-developed egg a) operculum and its connection with egg secured by means of teeth b) slight projection c) miracidium

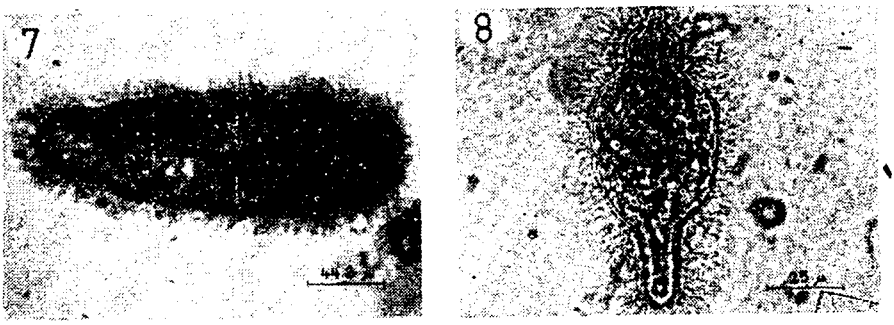


Figure 7. The miracidium
Figure 8. The miracidium at the beginning of the penetration process

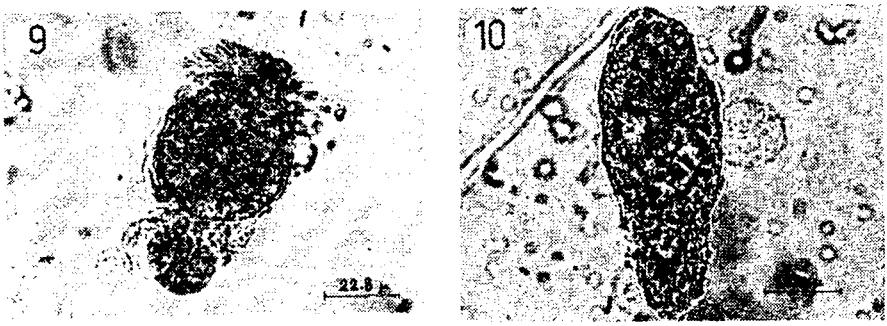
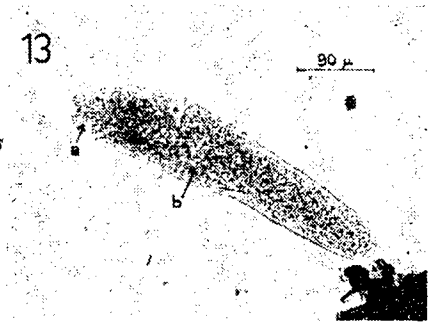


Figure 9. The miracidium one hour after the infection
Figure 10. The sporocyst two days after the infection

Figure 11. The sporocyst eight days after the infection
Figure 12. The mature sporocyst containing rediae 13 days after the infection



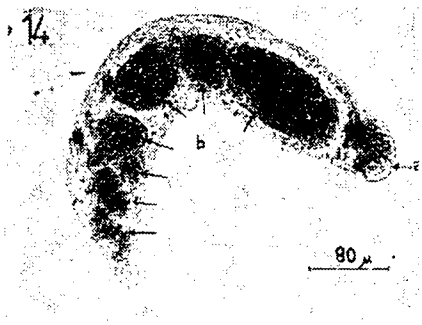
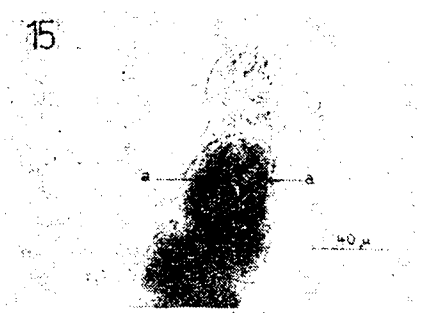


Figure 13. The young redia 14 days after the infection a) pharynx b) embryo balls
Figure 14. The redia containing the developing cercariae 22 days after the infection a) pharynx b) developing cercariae



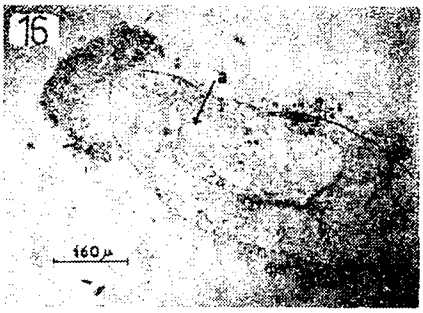


Figure 15. The anterior part of the mature redia containing the developing cercariae a) eye spots of cercaria
Figure 16. The anterior part of the mature redia containing the developing daughter redia. a) pharynx of daughter redia

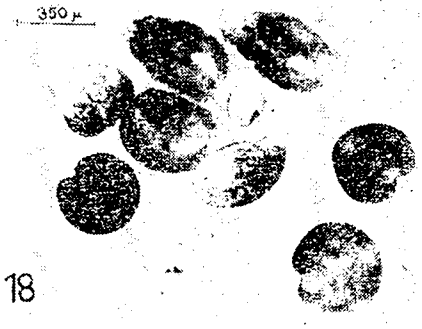


Figure 17. The small and short tailed young cercariae
Figure 18. Cercariae in different development stages

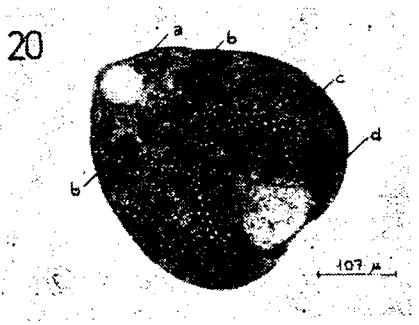
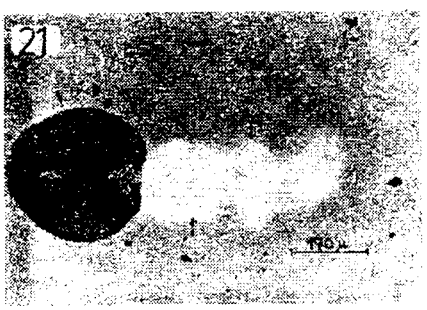


Figure 19. Cercariae which could be identified easily before P.planorbis dissection (pointed by arrows)

Figure 20. The body of the cercaria a) pharynx b) eye spots c) excreation ducts d) acetabulum



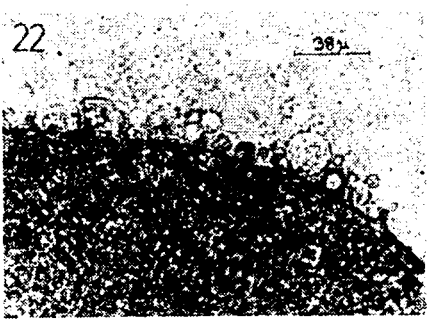
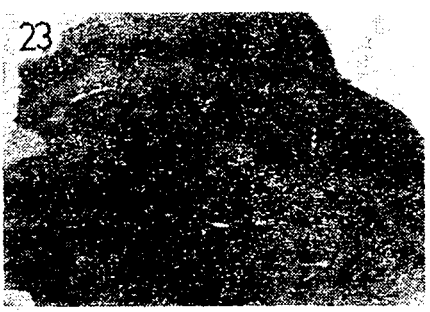


Figure 21. The mucoid fin around the tail of the cercariae

Figure 22. Cystogenous secretion during the process of encystment



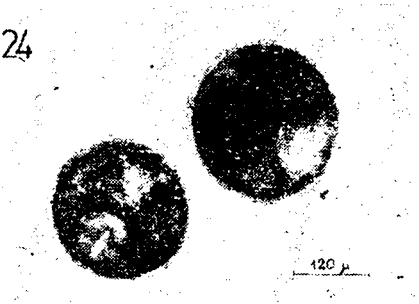


Figure 23. P.cervi metacercariae on the lettuce leave

Figure 24. The metacercariae