HISTOLOGICAL DETECTION OF FASCIOLA LARVAL STAGE IN SNAILS IN SULAIMANI- KURDISTAN- IRAQ

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ABSTRACT

Background Fasciolosis is an economically important disease of domestic livestock, in particular cattle and sheep, and occasionally man. The disease is caused by digenean trematodes of the genus Fasciola, commonly referred to as liver flukes. The two species most commonly implicated as the aetiological agents of fasciolosis are F. hepatica and F. gigantica (family Fasciolidae). F. hepatica has a worldwide distribution but predominates in temperate zones while F. gigantica is found on most continents, primarily in tropical regions.

Aims: Record the snail that is considered as intermediate host of fasciola and determine the most infected area by the snail that is mean infected area by fasciola and facilitates the control in human and animal fasciolosis.

Method: A descriptive crossectional study was undertaken at eight different regions in Sulaimany governorate diagnosed as snail sampling area that includes Penjewen/ Chamigawra, Sharazoor/ SaidSadiq, Saraisubhanaga, Penjewen/ ChamiNzara, Penjewen/ Homarsenan, Penjewn/ Hajieliasafa, Penjewen/ Kanichawazar and Sharbazher/ Khewata. Histological study of the snail was performed by the routine histological technique (the positive area was 8.3%, 8.6% and 10% respectively in Penjewen/Chamigawra, Sharazoor/SaidSadiq and Saraisubhanaga snails other regions was negative).

Conclusion: This is a new appearance of the incidence of fasciola in Sulamani province and the reason may be due to the climate and environmental changes that enhance parasite survival.

***Key words**: Fasciola, Lymnaea, snail. gigentica*

INTRODUCTION

Fascioliasis is caused by digenean trematodes related to genus *Fasciola* is a worldwide parasitic disease common in ruminants, especially cattle, buffaloes, sheep, goats, and swine. It may, however, affect humans (Marques and Scroferneker, 2003; Kelly *et al.,* 2004). Great economical losses are believed to be caused by such parasitism, leading to the decrease of meat and milk production as well as to high mortality rates in several countries in the world (Saleha, 1991 and Mendes *et al.,* 2008). The worldwide distribution of *F. hepatica* is related to climatic factors such as availability of humid habitats subject to flooding as well as the presence of definitive and especially intermediate hosts (Boray, 1969 and Dalton, 1999). The definitive host species in which the parasite develops plays an important role in determining the success of infection in the intermediate host (Vignoles *et al.,* 2004). Definitive host species eliminate, viable eggs in their faeces and the development of miracidia which occurs in the environment. The miracidium must then penetrate into a susceptible snail, the intermediate host. In the snail tissues the larvae undergo some changes, to develop to rediae and later to cercariae. Soon after their emergence from the host, the cercaria changes to metacercaria. To complete the life cycle metacercariae must be ingested by definitive hosts. Fasciola intermediate host snails belong to the genus Lymnaea. Lymnaeidae are widely distributed in fresh water (hermaphrodite pulmonata with a dextral shell and without operculum). There are over 20 species of the genus and many of them are *F. hepatica* and *F. gigantica* transmitters. (Saleha, 1991; de Souza *et al.,* 2002). Histological techniques have been widely employed to define the migratory routes of some parasites in their hosts and to study tissue alterations in snails due mainly to trematode infection (Kelly *et al.,* 2008). In these histological studies, samples

were usually fixed in formalin and embedded in paraffin. They were then histologically sectioned and stained with haematoxylin-eosin (HE). In developing countries little published information exists and data on the prevalence of helminthic infections, particularly on fascioliasis. Present study is the first one about detection of larval stage of liver fluke in the snail.

MATERIALS and METHODS

Samples collection

Between October and December 2007, one hundred and eighty-five samples were collected from eight different regions in Sulaimany governorate (100 being from *Lymnaea sp.*, 45 from *physa sp.* and 40 from *Gerolis sp.*).

Table 1: number of samples obtained from different regions.

Regions name	*No.of samples*	*Lymnaea Sp.*	*physa sp.*	*Gerolis sp.*
1- Penjewn/ChamiNzara	17	12	4	1
2- Penjewn/Kanichawazar	25	8	7	10
3- Penjewn/Homarsenan	25	13	9	3
4- Penjewn/Hajieliasafa	20	7	4	9
5- Penjewn/Chamigawra	30	24	2	4
6- SaidSadiq	29	23	5	1
7- Saraisubhanaga	15	10	4	1
8- Khewata	24	9	2	13
Total	185	106	37	42

Histological procedure:

The preparation of histological sections depended on standard methods of (Baker *et al.*, 1975; Leeson and Leeson, 1981) as follow:

The snail organ (after removing shell) was cut into small pieces with morphological abnormalities and put in a small container contained 10% formal saline and kept for 12 hrs for sample fixation.

The Specimens were dehydrated through a scending grades of more ethanol starting from 70%, 90%, and 100% twice, 3 hrs for each concentration and placed in xylene (most rapid clearing agent) for 2 hours. Specimens were thoroughly placed in the mixture of melted paraffin and xylene for 2 hours at 60 °C.

Moulds for Embedding:

The specimens were placed in a metal template filled with melted paraffin after embedded wax was solidified the paraffin blocks were removed from the metal and the individual paraffin blocks trimmed for preparing block with parallel sides.

Sectioning (Cutting the section):

This process was done by rotary microtome; the thickness was gauged at four microns, and then microtome operated until complete sections were again being cut and then maintained a regular cutting rhythm, and then the sections were affixed on slides.

Mounting the sections on slides:

Several slides should be cleaned, smeared with a drop of Mayer's egg albumin. Fixing the section on the slide by using a hot plate.

The slide was flooded with distilled water 55 °C , the sections were placed on the slide, ribbons of sections arranged on the slide and placed on hot plate, at 45 °C in order to stretches the ribbon and removing all creases. When the sections are fully extended, the slides were removed from the hot plate, drain off excess water and afterwards the sections stained by haematoxylin-eosin (HE).

The sections examined by light microscope under magnification power 40X. Photographs taken by computerized microscope-camera (GKBCCD color digital camera).

RESULTS

Percentage of larval stage:

From total snail number 185 only 106 were Lymnea spp and from total Lymnea spp only 5 was infected fig1.A total of 106 *Lymnaea* sp were included in the present study. These include 10 from Penjewen/ChamiNzara, 10 Penjewen/Kanichawazar, 10 Penjewen/Homarsenan, 10 Penjewn/Hajieliasafa, 20 Penjewen/Chamigawra, 20 Sharazoor/SaidSadiq, 10 Sharazoor/Saraisubhanaga and 10 from Sharbazher/Khewata were analyzed for the presence of larval stages (Redia or Sporocyst) specific for *Fasciola* using Histological Technique, of which 2 (10%) were Penjewen/Chamigawra, 1 (5%) at Sharazoor/SaidSadiq, and 1 (10%) Sharazoor/ Saraisubhanaga snails were infected with *Fasciola*. Overall infection rate was 4 (4%) (Table 2).

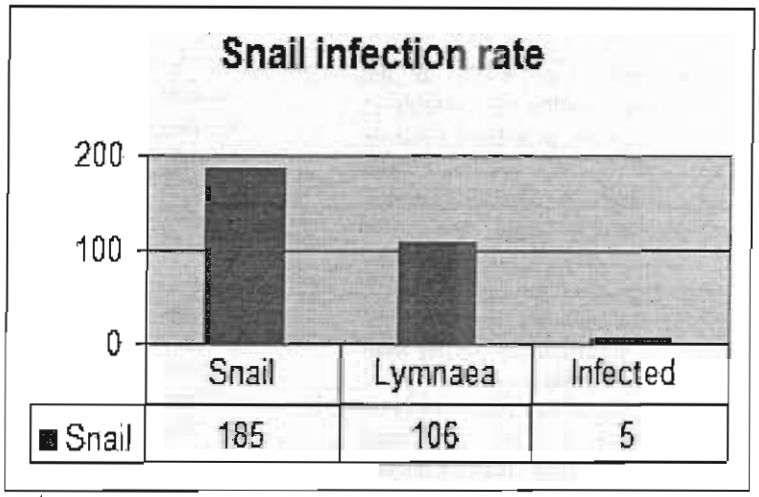

Fig.1 Snail infected rate

Table 2: Prevalence of fascioliasis in snails.

Samples area	*Lymnaea Sp.*	Positive Snail	(%)Percentage of Positive Snails
Penjewen/ChamiNzara	12	-	0
Penjewen/Kanichawazar	8	-	0
Penjewen/Homarsenan	13	-	0
Penjewen/Hajieliasafa	7	-	0
Penjewen/Chamigawra	24	2	8.3
Sharazoor/SaidSadiq	23	2	8.6
Sharazoor/Saraisubhanaga	10	1	10
Sharbazher/Khewata	9	-	0
Total NO	106	5	4.7

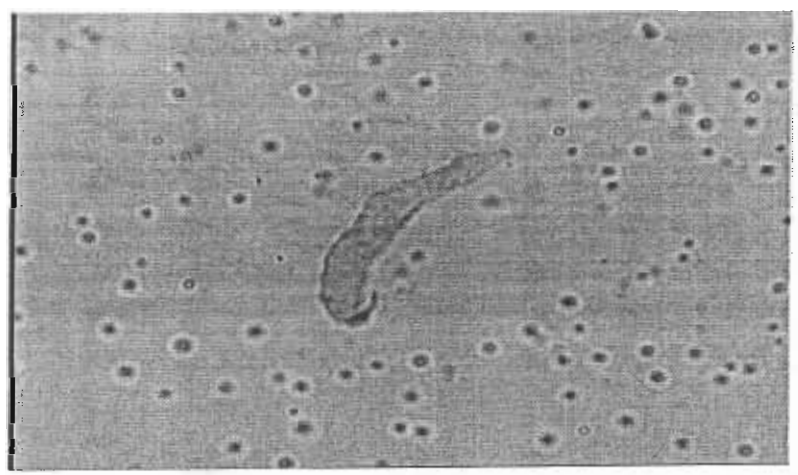

Fig.2: Histological slide HE stained, visualized by light microscopy showing *Lymnaea sp.* infected with redia.

Under light microscopy analysis, redias of *Fasciola* were found in the lumen of different segments of the digestive tract of *Lymnaea sp.* (Fig.2) The present study is the first of its record in this region.

DISCUSSION

Though the overall prevalence of 4.0% in the intermediate host in different location as in (table,1) is similar to prevalences in other countries such as Algeria (4.0%,), (*Mekroud et al.,* 2004), France (5.1%,), (Mage *et al.,* 2002). or Spain (11.4%,), (Manga-Gonzalez *et al.,* 1991) there are clear differences of prevelances in populations from the different types and locations of habitats. In reeds, wells and spring swamps the risk for snails to get infected with *F. hepatica* is significantly higher than in streams. Reeds and spring swamps are typical *L. truncatula* habitats. Silted wells also make an ideal habitat for a stable population of *L. truncatula.* Snail populations in reeds, wells and spring swamps do not undergo as much fluctuation, as populations in streams. Therefore, the infection risk for each snail increases by time. Furthermore, miracidia will more easily find snails in stagnant than in flowing water. From this it can be concluded that the potential risk of infection for cattle grazing near to reeds, spring swamps or on pastures with silted wells is higher than alongside of streams.

The failure to detect any *Fascola* larval stage in histological slides through light microscopy was also reported by Hodasi (Hodasi, 1972) and Lessa (Lessa, 2001), who pointed out the difficulties to find parasite larval stages in digestive gland lobules during regular intervals. However, histological alterations in the tissue adjacent to the stomach, digestive and albumen glands were observed. Degeneration and necrosis in tissues were usually observed in *Lymnaea sp.* infected by *Fasciola* due to mechanic trauma as a consequence of parasite movements from the feet to the digestive gland of the snail (Hodasi, 1972; Patnaik, and Ray, 1966). When the sporocysts are young and located in the cephalopodal region, or when the snails are collected in the field and probably infected with different trematode (Loker *et al.,* 1982), it is impossible to diagnose the trematode through those methods due to the similarity of young stages. Another difficulty arises when the snails collected in the field die before their arrival to laboratory, precluding any chance of trematode detection. Such remarks are quite important once the snails should be kept in laboratory and periodically observed, in order to obtain reliable results.

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الكشف عن يرقات الفاشيولا في القواقع بمحافظة السليمانية – اكردستان – العراق

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تعتبر ديدان الفاشيولا من الأمراض الهامة التي لها تأثير إقتصادى وتصيب الحيوانات الأليفة. وهنـاك نوعـان من الفاشيـولا وهما F. hepatica and F. gigantica وتعتبر F. gigantica الأكثر شيوعاً في معظم البلدان وخاصة في المناطق الحارة. إن هدف هذا البحث هو التسجيل والتعرف على القواقع (العائل الوسيط) وتحديد أكثر المناطق المصابة وبالتالي يسهل التحكم في انتشار المرض للإنسان والحيوان. تمت الدراسة ثمان مناطق بمحافظة السليمانية وذلك بأخذ القواقع وهى:

Penjewen / Chamigawra, Sharazoor /SaidSadiq, Saraiaubhanaga, penjewen /ChamiNzara, Penjewen /Homarsenan, Penjewn/Hajieliasfa,

استخلصت من هذه الدراسة أن ظهور الفاشيولا في محافظة السليمانية تعزى إلى التغيرات البيئية والحرارية التي قد تساعد على حياة الطفيل.