INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2005/07–6–928–931
http:// www.ijab.org

# Identification of *Schistosoma bovis* Through Mouse Model

ARIJO, A.G[1]., S. SAHITO, A.B. KALHORO, F.M. SOOMRO AND T.A. QURESHI
*Sindh Agriculture University, Tandojam, Pakistan*
[1]Corresponding author's e-mail: a_arijo@hotmail.com

## ABSTRACT

The research was carried out to identify the species of genus *Schistosoma*, which are digenic trematodes and parasitize animals and humans in 72 different countries of the world. The screening of 2006 *Biomphalaria spp.* snails collected from lentic and lotic waters of 14 different areas from Taluka Tando Allahyar, district Hyderabad revealed that 2.31% snails were naturally infected with schistosome cercariae. The month-wise screening of snails showed the schistosome infection at the rate of 1.15, 1.02, 3.89, 1.85, 3.65, 6.43 and 0.53% in the months of May, June, July, August, September, October and November, respectively; whereas, snails collected and screened in months of December, January, February, March and April did not shed any cercariae. In order to identify the species of schistosome, the cercariae collected and pooled from infection-positive snails were introduced to infection-free mice. The mice were left for 45 days to develop infection properly. The post-infection examination of shape and size of eggs confirmed *Schistosoma bovis* infection.

**Key Words:** *Schistosomiasis; Schistosoma bovis;* Mouse model

## INTRODUCTION

Schistosomes are flukes that parasitize humans, cattle, dogs and other animals in various parts of tropical and sub tropical countries including Asia, the Far East Africa, South America and the West Indies. In India, Pakistan and Bangladesh various species of Schistosome such as *S. nasalis, S. bovis, and S. mansoni* have been reported. In India and Bangladesh *S. nasalis* are relatively common and cause nasal granulomas in cattle and buffalo (Southgate & Agarwal, 1990).

*Schistosoma bovis* occurs in the portal and mesenteric veins of cattle, sheep and goats in Africa, the Mediterranean area Middle East and South East Asia. The parasite is also found in equines, camels, wild ruminants and rodents and occasionally in man but produces serious pathogenecity in cattle and sometimes sheep (Soulsby, 1982).

Research on Schistosomiasis has been ignored in Pakistan. There is one report on *S. mansoni* (a human schistosome) and very few reports about prevalence of' schistosomes of veterinary importance in Pakistan. Abdulssalam and Sarwar (1954) reported *S. spindalis, S. nasale* and *S. indicum* from various localities of Punjab province of Pakistan while Anwar and Gill (1990) reported 13% *S. bovis* natural infection in cattle and buffaloes in Raiwind area in same province. Arijo *et al.* (1999) screened snail collected from water bodies in and around Tandojam, Pakistan and reported that 7% of the snail were shedding schistosome cercariae.

However, the species of the genus Schistosoma were not known. This study was therefore conducted to diagnose the species that parasitize animals in Hyderabad Sindh, Pakistan.

## MATERIALS AND METHODS

The laboratory protocol used in this study was same as adopted by Arijo and Doenhoff (1997).

**Snail**

**Field collection.** The snails were collected from fresh water ponds and watercourses of 14 different villages of Taluka Tando Allahyar, district and division Hyderabad. The snails were collected through hand picking and net catching methods. The collected snails were placed in jars containing water. From that habitat the jars were brought to the Laboratory of Department of Veterinary Parasitology, Faculty of Animal Husbandry and Veterinary Sciences, SAU, Tandojam.

**Laboratory culture of snail host.** In laboratory, glass tanks with 12-L water holding capacity, were kept for the culture of intermediate snail host. The tanks were linked with controlled aeration system in order to oxygenate the water. Each tank was, however, filled with 10 L of water in order to prevent the snails crawling out. Randomly collected snails were kept in water filled glass tanks at room temperature and snails were fed *at libitum* everyday with commercial fish flakes.

Tanks were cleaned intermittently and any dead snail was removed from the tanks to prevent the degradation of water quality because dead snails are reported to decompose and foul the water rapidly at normal temperature (Webbe & Sturrock, 1964).

**Laboratory screening of snails.** The protocol used here was same as established by Webbe and Struck (1964).

**Method.** Approximately 5 mL of water was filled in small glass vials. In each vial, only one snail was placed gently and tubes were kept under lamplight at least for 60 minutes.

The light stimulates eruption of cercariae from the body of the snail (Arijo & Doenhoff, 1997). The vials were hold in front of light, where cercariae may be seen easily. The presence of cercariae was also confirmed under dissecting microscope. A drop of iodine was added in water to stop the movement of cercariae. The iodine also stains the cercariae hence they may be seen easily.

**Initial identification of cercariae.** Cercariae with bifurcate tail were identified as Schistosome cercariae.

**Mice**

**Maintenance.** Laboratory mice both male and female were maintained in-groups of 5-6 animals per cage, and were kept on wood shavings in every cage. Supply of drinking water (distilled) was properly given. Mice were fed *at libitum* on wheat flour mixed with chick-mesh prepared a fresh every day. Before reaching sexual maturity i.e. 4 weeks after birth, male and female mice were separated.

**Laboratory infection of mice.** The infection was given according to the "Ring Infection Method" originally described by Smithers and Terry (1965). According to this technique, the mice were anaesthetized by intra-peritonial injection of Ketamine HCL injection 100 mg $mL^{-1}$ a product of Fort Dodge Animal Health, Fort Dodge Iowa 50501 USA, at the dosage of 65-80 mg $kg^{-1}$ body weight and xylazine (a product of Phoenix, Pharmacutical, Inc, St: Joseph. Missouri). A dosage of 3-4 mg $kg^{-1}$ body weight was administered to anaesthetize each mouse. On becoming anaesthetized, the abdomen was shaved with shaver. The mice were laid on their backs and the shaved area moistened and cleaned gently with cotton wool soaked in water. Rings made of plastic or nickel were placed on the abdomen and held there by means of transparent adhesive tape. The adhesive tape was slightly punctured to allow the suspension of cercariae to be placed into ring on skin. Before applying, the suspension containing cercariae was gently mixed to distribute the cercariae evenly in order for an equivalent number to be given to each mouse. The cercarial suspension was then pipetted into the ring and mouse were exposed to cercarie for not less than 30 minutes in order to provide chance to cercarial penetration. The infected mice were placed in a warm place after exposure to infection to help them recover from the effect of anesthesia, after which the animals were returned to their cages.

**Fecal examination.** The fecal examination of infected mice was performed at least 45 days post infection.

**Method.** The mice were placed in the beaker for some time. Mice generally defecate as they are placed in beaker. The fecal sample was collected in the petri dish and a small quantity of water, was added to make a homogenous emulsion. The emulsion was added in the centrifuge tube, and centrifuged at 2000 r.p.m. for 2-3 minutes. The supernatant was discarded and the process was repeated twice. With the help of dropper, few drops of residues were picked from the bottom of tube and placed on the slide and covered with a cover slip.

The eggs were initially focused under 10x low magnification. Further structure was observed at 100x high magnification using oil immersion. The eggs were identified on the basis of their size and shape.

## RESULTS

The experimental model was designed to identify the schistosome species present in the area under study. The entire research model was categorically divided into five parameters hence the results together with observations are explained accordingly.

**1. Field collection of snail.** The snails were collected from lotic and lentic waters from 14 different villages of Taluka Tando Allahyar district Hyderabad, from January to December. From Table I, it may be concluded that (i) the potential intermediate snail host was prevalent in district Hyderabad, and (ii) the population of *Biomphalaria spp.* snail decreases in winter due to hibernation period observed by snails and it gradually increases in summer months.

**2. Screening of collected snails.** During this study, a total of 2,006 snails were collected. Out of that, 148 snails died before they were set to screen. Therefore a total of 1,858 snails were finally screened (Table I). This table further reveals that a total of 2.31% snails were Schistosome positive. It can be observed from this table that during the months of December, January, February, March and April, the snails were schistosome-negative, which indicates that the life cycle of the parasite is highly temperature dependent. Table I also reveals that the lowest rate of schistosome infection was in the month of November (0.53%) and highest in the month of October (6.43%).

**3. Pre infection mice condition.** As mention above, the purpose of the study was to identify Schistosome species by using a Mouse Model. The mice were reared under laboratory conditions but prior to introducing infective cercariae, all experimental mice were subjected to fecal examination. All mice were found negative for any parasite (Table II).

**4. Post-infection mice condition.** The parasite-tree mice were injected with schistosome cercariae intra-peritonially and left to develop infection for 45 days. The fecal samples were repeatedly collected from mice and thoroughly examined. Table III reveals that out of 72 mice (six mice per groups) 35% mice develop infection.

**5. Identification of species.** The fecal samples collected from 35%-infected mice were subjected to microscopic examination. On the basis of structure and size, the *Schistosoma bovis* species was identified.

## DISCUSSION

The study under taken confirms that Schistosomiasis is present in Pakistan. The screening of *Biomphalaria spp* snails from 14 different areas of Tando Allahyar, district Hyderabad have shown that as much as 2.31% of the snails were naturally infected with schistosome infection (Table I).

**Table I. Month-wise schistosome prevalence**

| Month | Total snails Collected | No dead snails before screening | Total screened | % positive |
|---|---|---|---|---|
| Jan | 34 | 5 | 29 | 00 |
| Feb | 32 | 2 | 30 | 00 |
| Mar | 55 | 9 | 46 | 00 |
| Apr | 166 | 9 | 157 | 00 |
| May | 289 | 29 | 260 | 1.15 |
| Jun | 211 | 15 | 196 | 1.02 |
| Ju1 | 244 | 13 | 231 | 3.89 |
| Aug | 243 | 27 | 216 | 1.85 |
| Sep | 255 | 9 | 246 | 3.65 |
| Oct | 241 | 8 | 233 | 6.43 |
| Nov | 207 | 21 | 186 | 0.53 |
| Dee | 29 | 1 | 28 | 00 |
| Total | 2,006 | 148 | 1,858 | 2.31 |

**Table II. Pre-infection fecal examination**

| Total mice examined | % positive parasite | % negative parasite | Spp: of parasite |
|---|---|---|---|
| 72 | 0 | 100 | Nil |

**Table III. Infection fecal examination**

| Total mice infected | % died | % -ve | % + ve | Spp. Of parasite |
|---|---|---|---|---|
| 72 | 50 | 15 | 35 | S. bovis |

As far as the presence of Schistosome infection is concerned, the findings of present study are in agreement with those of Arijo *et al.* (1999) who screened snails from Tandojam and its adjoining areas and reported 7% snails naturally infected with Schistosomes.

Anwar and Gill (1990) made the first mention of *Schistosoma bovis*, while they carried out coprological examination of cattle and buffalo in Raiwind area in Punjab-Pakistan. However, the identif1cation of schistosome species through establishment of parasite life cycle in mouse as definitive host, and snail as intermediate host is first in its nature in Pakistan. Although there is difference of experimental protocol and area of study, but our results are in agreement Anwar and Gill (1990) with regard to the prevalence of *Schistosoma bovis* in Pakistan.

It was a common belief in the first half of the century that Schistosomiasis would not become establishes in the Indian sub-continent (Southgate & Agarwal, 1990). The fact is that, both Pakistan and India have had contacts for centuries with countries such as Japan, China and Egypt, all of which have endemic Schistosomiasis (*S. japonicum* in Japan & China; *S. mansoni & S. haematobium* in Egypt). During the past century, wars have resulted in the movement of troops to various African and Asian countries and it is not surprising that some troops on their return may have carried active schistosome infection.

All schistosomes need snails as their intermediate hosts to complete the asexual phase of their life cycles. None of the intermediate host species was found in India, nor were any endemic snails were found to be compatible with human schistosome (Chauhan & Cauhn, 1957; Baugh, 1978).

It was also believed that *S. haematobium* and *S. mansoni* may only develop in humans and can not develop in other animals; like wise, it was a widely held view that schistosomes which infect animals cannot develop in humans. In the light of later research (Rollinson & Southgate, 1987) reported that *S. mansoni* and *S. haematobium* have a wide range of potential host, and *S. haematobium* is not restricted to humans.

Gadgill and Shah (1952) reported about 250 people passing *S. haematobium* shaped eggs in their urine in Gimvi village in India. Khalil *et al.* (1994) diagnosed *S. mansoni* from 7 years old girl from Karachi, Pakistan. The patient was a non-vegetarian and had no pets at home, nor had she traveled to any other region in the recent past. Bidinger and Crompton (1989) reported the findings of terminal-spined schistosome eggs from two stools and two urine samples originating in the environs of Dokur, Andhrapardesh, India.

There are a few reports about prevalence of schistosomes of veterinary importance in Pakistan and India. Anwar and Gill (1990) examined a total of 20,000 animals from different localities of Punjab province of Pakistan and 13% cattle and buffaloes infected with *S. indicum* and *S. bovis*. Abdulssalam and Sarwar (1954) reported *S. spindalis, S. nasalis* and *S. indicum* from various localities of Punjab province of Pakistan.

In India, *S. nasalis* has been reported from Andhrapardesh (Rao & Murthy, 1964; Christopher & Rao, 1976; Sreeramula, 1982), Bihar (Varma, 1954; Sahay & Sahai, 1979) Karnatak (Naik, 1942; Rao & Naik, 1957; Muraledhran *et al.*, 1976 a, b, d).

The role of reservoir hosts and of strains of the parasite has importance as epidemiological factors, depending on the species. Members of no less than seven mammalian orders have been successfully infected experimentally with *S. mansoni;* however, certain monkeys and a variety of rodents are probably important natural reservoir hosts in Africa and tropical America. *S. haematobium* is more host specific than *S. mansoni*, and it is thought that no natural reservoir hosts exist for it. The opposite is true of *S. japonicum*, which seems to be the least host specific. It can develop in dogs, cats, horses, swine, cattle, rodents and deer. However, there seems to be more than one race of this worm, and the susceptibility of a given host varies. For example *S. japonicum* is widely prevalent in rates in Taiwan, but is rare in humans there. The recent description of *S. mekonji* and *S. malaganum* as a distinct from *S. japonicum* suggest that the traditional *S. japonicum* may be a complex of cryptic species (Roberts & Janovy, 1996).

An unusual snail host has been reported for *S. haematobium* in Portugal where *planorbarium metidjensis* has been regarded as responsible for transmission of this parasite (Azvedo, 1969). However it seems, possible that *P. metidjensis* may be only an occasional host and of secondary importance to *B. truncates*, which also occurs in

Portugal.

In the Indian sub-continent several species of fresh water mollusks are found including *Indoplanorbis exustus, Lymnae luteola, Paludomus obesa, Ferressia tenuis* and *Melanoides tuberculata.* Gadgill and Shah (198S) expressed numerous snail species to miracidia originating from a patient with urinary Schistosomiasis (*S. haematobium*) from Gimvi village of India and the only specie they found to shed schistosome cercarie despite heavy mortality, was the very small ancylid snail *F. tenuis.* In an examination of 1, 200 *F. tenuis* Sathe and Remapuker (1983) found only 12 specimens to be infected with schistosome cercarie. On this evidence it is generally assumed that *F. tenuis* is responsible for transmission.

The snail fauna of Pakistan is quite rich and many species are involved in transmission of parasitic infections. The present study has shown that the *Biomphalaria spp* snail is certainly transmitting the *S. bovis* infection. It is interesting to mention that, a good number of *Biomphalaria spp* snails were found to shed *Fasciola hepatica* cercariae alone, while some snails were found to shed both Fasciola and Schistosome cercariae at same time, which indicates that snails may allow different species of parasite to development their life-cycle. This phenomenon helps to suggest that a large-scale study on various species of snails may be conducted to obtain the data on host-parasite relationship diversity.

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**(Received 20 March 2005; Accepted 28 June 2005)**