STUDIES ON INCIDENCE AND CHEMOTHERAPY OF *Schistosoma indicum* IN SHEEP

BY

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HISAR

2000

DEDICATED

TO

MY REVEREND PARENTS

CERTIFICATE - I

This is to certify that this thesis entitled, "**Studies on incidence and chemotherapy of *Schistosoma indicum* in sheep**", submitted for the degree of M.V.Sc. in the subject of Veterinary Parasitology to the CCS Haryana Agricultural University, is a bonafide research work carried out by Dr. Devender Singh under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE - II

This is to certify that this thesis entitled, "**Studies on incidence and chemotherapy of *Schistosoma indicum* in sheep**" submitted by Dr. Devender Singh to the CCS Haryana Agricultural University in partial fulfilment of the requirements for the degree of M.V.Sc. in the subject of Veterinary Parasitology, has been approved by the student's Advisory committee after an oral examination on the same.

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HISAR (DEVENDERSINGH)

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1. INTRODUCTION

Sheep population (56.47 millions) of India ranks sixth in the world (FAO, 1999). The sheep rearing by landless labourers and marginal farmers in our country is unscientific which leads to number of bacterial, viral and parasitic diseases particularly helminthic infections. Helminths are well known enemies of sheep (Minnet, 1950) and have not attracted adequate attention of the scientists probably due to reduced mortality. In a tropical country like ours, helminths play more havoc than bacterial and viral diseases. The diseases resulting from them are often overlooked but their ill effects undermine the health of sheep and in turn lower their efficiency and output (Sircar,1956).

Among helminthic diseases, schistosomosis in India is now well recognised as a disease of great economic importance. Schistosomes viz. *Schistosoma indicum* Montgomery,1906; *S.spindale* Montgomery,1906; *S. incognitum* Chandler,1926; *S. nasale* Rao,1933; *Orientobilharzia bomfordi* Montgomery,1906; *O. turkestanicum* Skrjabin,1913; *O. dattai* Dutt and Srivastava,1952 and *Bivitellobilharzia nairi* Mudaliar and Ramanujachary,

1945 have been identified to infect one or other species of domestic animals. This disease is responsible for reduced body weight gain, reduced conception and pregnancy rates, increased age at puberty (Mc Cauley *et al.*, 1983; Dargie, 1980) and poor production of mutton , wool and milk (Srivastava *et al.*, 1964). It also leads to liver condemnation (Rao, 1947; Dargie,1980).

Schistosoma indicum, the cause of hepato-intestinal and pulmonary schistosomosis, in animals was first reported in equines (Montgomery,1906) of India. Later, it has been recorded from sheep, goats, cattle, buffaloes, camels and elephants in several states of India (Datta, 1933; Rao,1947; Satyanarayanacharyulu *et al.*, 1969; Banerjee and Agrawal, 1989 and 1992; Rajkhowa *et al.*, 1992; Chaudhri *et al.*, 1994; Sharma and Dadhich, 1998). Other than India , this schistosome species has also been recorded in sheep and equines of Pakistan (Bhalerao,1932 ; Datta,1933) and cattle and goats of Bangladesh (Islam,1975).

Schistosomosis in sheep is characterised by blood tinged foetid diarrhoea, anorexia, anaemia and loss of condition leading to death after an illness of 2-3 weeks (Singh *et al.*,1985). Pulmonary infection of *S. indicum* in sheep has been found associated with general weakness, loosening of wool , loss of body weight , laboured respiration, rhinitis and oedema around nostrils of upper lip (Lodha *et al.*,1981).

Accurate assessment of any disease warrants the use of sensitive diagnostic methods. Demonstration of schistosome eggs in faeces is a confirmative diagnostic test. Sieving method described by Soulsby (1982)

and subsequently modified by Banerjee and Agrawal (1989) has been commonly used to detect *S. indicum* eggs in faecal samples. Schistosome eggs contain fully developed miracidia when passed out in the faeces. These eggs hatch after coming in contact with water and this characteristic was used in developing a hatching technique for their detection in faecal samples. This technique has been found more sensitive than sieving method (Dutt and Srivastava ,1961; Agrawal and Panesar ,1987). Liver biopsy has been practised since long in man for detecting schistosome eggs particularly in chronic infections. However, it has rarely been tried in Veterinary practice. Nevertheless, its modification has been used by taking liver tissues from slaughtered animals (Rao,1947; Nara and Nayak,1972). This method has been found more sensitive in detecting schistosome eggs in slaughtered animals (Banerjee and Agrawal, 1989).

Till date control of schistosomosis has been attempted by using biological means (Jorden *et al.*,1980; Madsen ,1990), chemotherapy (Chaudhri *et al.*,1994; Johansen *et al.*, 1996), heterologous immunity (Massoud and Nelson , 1972), irradiated vaccines (Bushara *et al.*,1978 ; Majid *et al.*,1980), crude schistosome antigens (Aradaib *et al.*,1993), whole egg antigens (Araidaib *et al* .,1995) and defined protective antigens (Bushara *et al.*,1993). Among them anthelmintic control has remained practical method under the circumstances that till now no vaccine is available for field use .

Lithium antimony tartrate, which was found effective in controlling *S. indicum* infection (Supekar and Jain 1977; Lodha *et al.,* 1981; Singh *et al.*, 1985) has doubtful efficacy (Chaudhri *et al.*, 1994; Srivastava and Agrawal,1999). Praziquantel with a high therapeutic efficacy, easy to administer and well toleration is now recognised as an antischistosomal drug of choice (Davis,1993). It has been used successfully in calves and goats against *S. bovis* (Bushara *et al.,* 1982 ; Johansen *et al.*, 1996), *S. spindale* (Upatoom *et al.*, 1988) and *S. nasale* (Anandan and Raja , 1987). Till date praziquantel has not been tried against natural infection of *S. indicum* therefore, it was considered useful to evaluate this drug in sheep .

Despite impressive studies on various aspects of schistosomosis due to *S. indicum,* it is essential to study the incidence pattern and chemotherapy of this disease in sheep of Haryana where it is one of the emerging diseases. Keeping in view the above facts, present study was planned with following objectives :-

1. To determine the incidence of *S. indicum* in sheep of Karnal district (Eastern Haryana).
2. To evaluate the chemotherapeutic efficacy of praziquantel in sheep against *S. indicum* .

2. REVIEW OF LITERATURE

Schistosomosis of man and animals is mainly a problem of tropical and subtropical zones of world. The causative parasite belongs to the family schistosomatidae (Trematoda) which dwells in blood vascular system of the host. None of the schistosome occurring in Asian countries and causing endemic form of schistosomosis in domestic animals have natural distribution outside Asia and in this context the situation presents a geographical entity itself (Kumar and de Burbure, 1986).

Though extensive literature is availabe on schistosomes and schistosomosis, yet most part of this concerns with human schistosomes and their experimental infections in laboratory animals. Comparatively less work has been done on animal schistosomosis. Out of this a large portion has been contributed from African countries (Lawrence,1978; De Bont and Vercruysse,1998). The schistosome species prevalent in these countries are different from those found in India. The pertinent literature dealing with different species has also been included in this review to have a

comprehensive view on incidence and chemotherapy of animal schistosomosis particularly due to *Schistosoma indicum*.

2.1 Incidence

In 1882, Cobbold reported occurrence of schistosomosis in cattle and sheep for the first time in India. About two decades later Montgomery (1906) studied animal schistosome species for the first time and in his pioneer work he dealt with five species of genus *Schistosoma* including *S. indicum*, which was a new species recorded in Indian horses and donkeys. The lesions associated with heavy infections of this schistosome were enlarged, uneven, rough and firm livers with pseudotubercles varying from pin head to pea size.

Bhalerao (1932) recorded *S. indicum* for the first time from cattle and goats at Imperial Institute of Veterinary Research, Muktesar.

Datta (1933) gave an exhaustive histological description of lesions caused by *S. indicum* in equines. *Schistosoma indicum* was reported from 9 specimens obtained from horses and mules of Rawalpindi, Lucknow, Secundrabad, Ambala, Queta and Mhow military establishments.

While working on Kumri, a serious chronic disease of horses, Malkani (1933) suggested that it might be nothing but a form of equine schistosomosis. The identification of the schistosomes recovered from died horses resembled to *S. indicum* (Montgomery, 1906).

Rao (1947) examined 2000 sheep slaughtered at small abattoir of Poona R.I.A.S.C. butchery and found 436 (21.8%) livers harbouring *S. indicum* eggs.

Murthy (1950) reported *S. indicum* eggs in three bovine faecal samples. The subsequent faecal examination of 60 cattle, 4 sheep and 7 goats yielded *S. indicum* infection in 9 cattle only. The aquatic snails, *Indoplanorbis* spp., at that location were also found harbouring schistosome cercariae.

Verma (1954) reported overall incidence of schistosomosis to be 0.2% at Govt. Livestock Breeding Farm, Patna.

Raghwan (1958) recorded nodular cirrhosis of liver in equines caused by schistosomes. Out of 15 ponies examined, 8 showed characteristic lesions and 4 showed less conspicuous lesions of schistosomosis.

Bhatia (1960) while studying liver affections with three species of fluke parasitizing Indian sheep, gave a short account of lesions produced by *S. indicum* in naturally infected sheep.

Rai (1960) examined large number of carcasses to study incidence of natural infection of *S. indicum* in equines and found heavy infection with *S. indicum* in local ponies and donkeys on a number of occasions.

Dutt and Srivastava (1961) developed a new method of identifying schistosome species by studying epidermal structure of recovered miracidia from faeces. Hatching technique was advocated over egg detection for diagnosing schistosomosis because of higher sensitivity of the former.

Srivastava and Dutt (1962) prepared a monograph on morphology, life history, intermediate hosts and definitive hosts of *S. indicum* on the basis of their comprehensive study. Their studies suggested that sheep and goats were primary natural hosts whereas cattle and buffaloes were minor hosts.

Endrejat (1964) reported from Assam that intestinal schistosomosis was caused by *S. indicum* and *S. spindale* in ruminants.

Sayal (1964) reported from Jabalpur (M.P.) an overall incidence of *S. indicum* in 37% of ovine livers.

Srivastava *et al.* (1964) conducted an experiment to determine the pathogenicity of *S. indicum* infection in young sheep. Six animals were exposed to 7,000-15,000 cercariae and another six served as control. The experimentally infected sheep had higher mortality, highly significant reduction in weight gain, haemoglobin levels, erythrocytes, leukocytes and a significant increase in eosinophils.

Arora and Iyer (1968) collected representative samples of liver from sheep and goats slaughtered in Municipal slaughter-house and Military slaughter-house, Bareilly and found 26.3% livers infected with *S. indicum*.

Satyanarayanacharyulu *et al.* (1969) reported *S. indicum* infection for the first time, in an elephant at Tirupati (A.P.).

Jain (1972) examined 100 livers from military and civil slaughter-houses, Mhow and Burhanpur and reported that *S. indicum* infection was present in 10% of them.

Nara and Nayak (1972) from Bhubeneswar (Orissa) recorded *S. indicum* infection in 107 out of 220 sheep livers showing various macroscopic changes. They opined that schistosomosis resulting from *S. indicum*, constituted a major pathological condition in livers of sheep and goat.

Islam (1975) examined viscera of 224 cattle, five buffaloes, 118 goats and 20 sheep from nine abattoirs situated throughout Bangladesh and recovered *S. indicum* from 96 cattle (43%) and 11 goats (9%). The overall incidence of visceral schistosomosis was 62% in cattle, 100% in buffaloes, 12% in goats and 10% in sheep. The prevalence and intensity of schistosomes was also reported to increase with the age of animals.

Sharma and Dwivedi (1976) recorded pulmonary schistosomosis forming pseudotubercles in lungs of six sheep and two goats naturally infected with *S. indicum*. Multiple granulomas around schistosome ova were also observed.

Supekar and Jain (1977) reported a clinical case of *S. indicum* infection in a Gir bullock of Kasturba Gram Krishi Kshetra, Indore. Animal was found dull, depressed, completely off feed and passing small quantity of foul smelling faeces with a lot of mucous, blood and air bubbles.

Lodha *et al.* (1981) described an outbreak of pulmonary schistosomosis due to *S. indicum* in 3200 sheep at Daudsar village (Bikaner) of Rajasthan. The affected sheep had general weakness, loss of body weight and wool with 21% morbidity and 8% mortality. The lung sections had 7 to 12 *S. indicum* ova per low power microscopic field.

Vashishta *et al.* (1981) recorded a new focus of this parasite in Daudsar village (Rajasthan) where it caused an outbreak in sheep. The clinical observations made by them included dullness, progressive emaciation,

cachexia and respiratory signs in all the sheep. The digestive disorders (anorexia and dysentery with mucous and blood) were seen in 27% of the infected flock.

Agrawal and Sahasrabudhe (1982) found *S. indicum* eggs in liver and rectal scrappings of 17 (54.8%) cattle and 9 (26.4%) goats. Mixed infection of *S. indicum* and *S. spindale* was recorded in 9 (29%) cattle and 2 (5.8%) goats.

Bhaskararao *et al.* (1984) identified *S. indicum* the cause of debility, diarrhoea and death in 11 sheep from Karim Nagar district of Andhra Pradesh.

Mohan Rao and Choudary (1984) studied the incidence of schistosomosis by examining a total of 2773 tissue material from 6328 sheep and found 44 livers (0.75%) and 2 lungs (0.03%) positive for *S. indicum* eggs. The incidence of this infection was reported higher in the months of August to March.

Singh *et al.* (1985) reported a severe outbreak of visceral schistosomosis in two flocks of 140 and 40 sheep each in Kasoli village of Haryana. Of these 50% sheep died exhibiting clinical signs like blood tinged foetid diarrhoea, anorexia, anaemia and loss of condition leading to death after an illness of 2-3 weeks. The post-mortem examination revealed large number of *S. indicum* worms in mesenteric veins.

Singh and Parihar (1988) reported that on histopathological examination of 227 (4.09%) grossly abnormal livers from 5544 animals

(1164 sheep and 4380 goats), 21.59% had *S. indicum* infection. The livers having *S. indicum* eggs were hard, nodular and enlarged.

While studying the sensitivity of liver examination vis-a-vis faecal examination, Banerjee and Agrawal (1989) evaluated some diagnostic techniques viz. hatching technique, sieving method and liver press method. Liver press method was found more sensitive in diagnosing hepato-intestinal schistosomosis. Out of 50 cattle and 15 buffaloes examined, *S. indicum* eggs were detected in 27 (54%) cattle and 9 (60%) buffaloes by liver press method, 22 (44%) cattle and 6 (40%) buffaloes by hatching technique and 10 (20%) cattle and 2 (13.3%) buffaloes by sieving method. The sensitivity of liver press method was found to be 96.43% and that of hatching and sieving method were 78.57 and 35.71%, respectively.

In an epizootiological study, Banerjee and Agrawal (1992) reported that 88 (44%) cattle and 119 (38.4%) buffaloes were positive for schistosomes by hatching test whereas sieving method could detect only 34 (17%) cattle and 26 (8.39%) buffaloes infected with schistosomes.

Rajkhowa *et al.* (1992a) in Assam examined lungs, livers and mesenteries from 185 cattle, 110 goats and 25 buffaloes and found 27, 1.8 and 4% of them infected with *S. indicum*, respectively. The faecal examination of 323 cattle and 15 buffaloes revealed 12.4 and 0% infection of *S. indicum*, respectively. The overall incidence of *S. indicum* increased from August (35.29%) to February (42.85%).

Rajkhowa *et al.* (1992b) studied haematological changes in cattle experimentally infected with *S. indicum* and observed haemoglobin (Hb g%), packed cell volume (PCV %) and total erythrocytic counts (TEC million/ cmm) decreasing significantly from day 14 post infection (PI) to day 70 PI.

Vyas *et al.* (1992) studied hepatic vascular lesions in caprine schistosomosis and reported that 102 (9.87%) out of 968 livers from 3045 goats of either sex slaughtered at various abattoirs of Bikaner had characteristic lesions of *S. indicum.*

Chaudhri *et al.* (1994) conducted a prevalence study of *S. indicum* in sheep of Eastern Haryana and reported that sheep (33.2%) and goats (47.8%) were more infected than cattle, buffaloes and horses (12.5-16.6%). Sheep and goats were found to exhibit clinical signs of schistosomosis with 10-20% mortality.

Singh *et al:* (1995) identified pneumonic lungs in 351 (10.61%) of 3308 sheep and 249 (6.06%) of 4108 goats slaughtered at abattoir of Mathura. *Schistosoma indicum* was identified in pneumonic lungs of 19 (5.41%) sheep and 23 (9.23%) goats.

Dadhich and Sharma (1996) examined lungs of 2217 goats of either sex, age group and breed and out of 571 lungs showing gross abnormalities, 12 (2.10%) had pulmonary schistosomosis due to *S. indicum.*

Sharma and Dadhich (1998) from Bikaner, reported schistosomosis in camel without mentioning Schistosome species.

Khan *et al.* (1999) carried out epidemiological studies on ovine gastrointestinal parasitism in field conditions in semi-arid regions of Rajasthan and reported 6.9% incidence of schistosome infection.

2.2 Chemotherapy

World Health Organisation has taken keen interest in the disease schistosomosis under its Tropical Disease Research Programme and have been instrumental in controlling this disease in human beings by developing very effective drugs like praziquantel, oxaminiquine and metrifonate. However, these drugs have still not been tried extensively in animal schistosomosis.

In contrast to vast research efforts dealing with chemotherapy of human schistosomosis, the literature regarding chemotherapeutic trials against hepatointestinal and pulmonary schistosomosis in ruminants is scanty and has been reviewed as follows :

Dutt *et al.* (1963) reported that potassium and sodium antimony tartrate (@ 8.4 to 12.3 mg/kg body weight intravenously) as 10 doses were highly effective against *S. indicum* in naturally and experimentally infected sheep.

Supekar and Jain (1977) successfully treated a Gir bullock infected with *S. indicum* by administering lithium antimony thiomalate intramuscularly (i.m) for three consecutive days as per recommended dose rates.

Lodha *et al.* (1981) used two intramuscular injections of lithium antimony thiomalate (Anthiomaline @ 2.5 ml per sheep) on alternate days

in 100 sheep naturally infected with *S. indicum* where 16 sheep died inspite of the treatment. Later, they administered intravenous injections of 5 ml of 1% solution of potassium antimony tartrate on three successive days in 325 infected sheep. This resulted in recovery of all except 93 sheep which died from hepatic failure and pulmonary insufficiency.

Vashishta *et al.* (1981) treated the sheep infected with *S. indicum* with 6% (w/v) aquous solution of lithium antimony thiomalate (Anthiomaline @ 2.5 ml/sheep i.m on three occasions at an interval of 72 hr). Liver extract and vitamin B complex were given as supportive therapy. The sheep responded well to the treatment.

Bhaskararao *et al.* (1984) treated successfully 20 sheep naturally infected with *S. indicum* by administering lithium antimony thiomalate.

Singh *et al.* (1985) used lithium antimony thiomalate parenterally as per recommended doses along with supportive mineral therapy in sheep infected with *S. indicum.* and reported a good response to this treatment.

In contrast to observations of many workers (Supekar and Jain, 1977; Lodha *et al.*, 1981; Vashishta *et al.*, 1981; Bhaskararao *et al.*, 1984 and Singh *et al.*, 1985) on successful treatment with lithium antimony thiomalate, Chaudhri *et al.* (1994) reported that this drug eliminated *S. indicum* eggs completely on day 7 post treatment (PT) but on day 28 PT, a few of treated animals had eggs in their faeces. They further observed that this drug was unable to show clinical improvement in infected and treated sheep.

Chaudhri *et al.* (1994) also used ivermectin @ 200 µg/kg body weight subcutaneously in sheep naturally infected with *S. indicum*. The drug appeared to be of little use as only 20-40% reduction in faecal egg counts was recorded.

Later on Srivastava and Agrawal (1999) also evaluated efficacy of lithium antimony thiomalate in experimental schistosomosis due to *S. spindale* in guinea pigs. They reported that at double the normal dose, only 11.36% and 39.13% fluke reduction was observed in animals treated on day 90 and on day 120 PI, respectively. They suggested that lithium antimony thiomalate was not effective for chemotherapy of animal schistosomosis.

A lot of work seems to have been carried out on the use of praziquantel against schistosome species resulting into hepato-intestinal schistosomosis in ruminants of countries other than Asia. It has been reviewed to have comprehensive view regarding latest development on chemotherapy of animal schistosomosis.

The first report on activity of praziquantel against schistosomes in ruminants was made by Bushara *et al.* (1982) who treated four young cattle affected with *S. bovis* by using this drug @ 20 mg per kg body weight on week 9 and week 14 PI. There was 98.9% reduction in fluke recovery on week 16 PI.

Li-ZD *et al.* (1984) showed that single dose of 30 mg per kg body weight orally or 15 mg per kg body weight by omasal injection of

praziquantel was effective in treating schistosomosis in sheep and cattle in China.

Markovics *et al.* (1985) while examining the efficacy of praziquantel in *S. bovis* infections reported that this was the drug of choice when used as a single dose of 20 mg per kg body weight. However, its high cost limits its use in large ruminants.

Upatoom *et al.* (1988) used praziquantel for the treatment of calves naturally infected with *S. spindale* infection. The drug was given at the dose rate of 20 mg/kg body weight either orally or intramuscularly. Oral administration resulted into complete elimination of eggs from faeces by 60th day PT Egg output also decreased by intramuscular route but drug was not as effective as by oral route.

Markovics *et al.* (1993) reported two outbreaks of *S. bovis* in cattle and sheep and found that praziquantel @ 20 mg/kg body weight was a highly effective drug of choice for the treatment of *S. bovis* infection.

Johansen *et al.* (1996) treated experimental *S. bovis* infection in 15 goats by using praziquantel @ 60 mg/kg body weight and recorded per cent efficacy as 100, 99.4 and 95.7 on day 1, week 1 and week 4, respectively. They further observed a faecal egg count reduction of 84% and 93% on week I and on week 3 PT, respectively. The tissue egg counts also reduced significantly in intestines. However, the liver of treated animals had non-significant reduction in egg counts.

Quin Cheng Gui (1996) while examining efficacy of praziquantel against bovine schistosomosis reported that this drug was safe and highly efficient with a short course and simple administration method. The best time suggested for treatment of bovine schistosomosis was early winter (late November) to avoid reinfestation.

3. MATERIALS AND METHODS

3.1 Incidence data

Liver and faecal samples of 25-30 sheep from Municipal Committee slaughter- house, Karnal and faecal samples of 50 sheep from two selected flocks of area surrounding Parasitological Research Center, CCS HAU, Regional Research Station, Karnal were examined monthly for the presence of *S. indicum* eggs/miracidia. The information regarding age, sex and breed was gathered either from owner or at times personal assessment was made.

3.1.1 Field data

Fifty sheep, 6 months to 2 year old of either sex from two selected flocks in villages nearby Karnal were faecal examined monthly from August 1999 to July 2000 at Parasitological Research Center, CCS HAU, Regional Research Station, Karnal. The sheep under study subsided on grazing around ponds, banks of canals common village land, agricultural fields in post harvesting period, sides of roads and other uncultivated land. The stocking rate was high and animals grazed from morning till evening with slight break at noon. No extra feed was provided to them during night. Deworming was not practised as a matter of routine.

3.1.2 Slaughter-house data

Rectal faecal samples and liver pieces (5-10 g) were collected monthly from 25-30 sheep killed at Municipal Committee slaughter-house, Karnal from December, 1999 to July, 2000. The faecal samples were examined by sieving and hatching techniques for presence of *S. indicum* eggs/miracidia. The liver pieces were examined under stereoscope for presence of *S. indicum* eggs.

3.1.3 Assessment of infection

The parasitological methods employed were as follows :

Liver press method

This was performed following the method of Banerjee and Agrawal (1989) with slight modifications. In brief, representative liver pieces (5-10g) were collected from killed sheep. Thin pieces of liver sample were pressed between two microscopic glass slides and examined under stereoscope for presence of *S. indicum* eggs.

Hatching technique

It was carried out as per technique of Kaur (1985) with some modifications. In brief, 10 g of fresh faecal samples from each animal were homogenised with normal saline solution. The resulting sediment was mixed with clean water in a flask covered with carbon paper except at neck. It was exposed to artificial light for 3 hr. The material (10 ml) from neck of the flask was removed and examined under stereoscope for schistosome

miracidia. The miracidia were identified on the basis of their typical shape, size and movements.

Sieving method

This was performed as per method of Soulsby (1982) and further modified by Banerjee and Agrawal (1989). In the present study, 5 g of faeces was dissolved in 50 ml of 0.4N sodium hydroxide in 1.7% saline and kept overnight. Ten ml sample from the above suspension was taken and filtered through a normal tea strainer. The filterate was allowed to stand for 20 min and the supernatant was discarded. The sediment was diluted upto 30 ml using NSS and 3 ml sample (0.15 ml in each chamber of Mc Master slide) was examined under 50X magnification for presence of *S. indicum* eggs. The number of eggs present were multiplied by 10 to obtain EPG.

3.2 Chemotherapeutic trial

Praziquantel (Cysticide 500 mg Tablets, Bayer, India) @ 25 mg/kg body weight was used as a single dose for chemotherapy of sheep naturally infected with *S. indicum*.

3.2.1 Animals

A flock comprising of 100 sheep was screened for presence of *S. indicum* infection. Out of them 10 sheep about 1-2 year old of either sex with high EPG counts were purchased locally and numbered for identification. They were kept for experimental study under parasite free conditions on pucca floored animal house of CCS Haryana Agricultural University, Regional Research Station, Karnal. The animals were maintained

under hygienic conditions for 10 days before actual start of experiment. Ad-lib feed and water was provided to them and a constant vigilance on experimental animals was kept throughout the study.

3.2.2 Experimental design

The naturally infected sheep (10) were divided into two equal groups, infected treated (Gr. I) and infected untreated control (Gr. II) on the basis of EPG counts. Sheep of Gr. I were treated with single dose of praziquantel @ 25 mg/kg body weight orally while sheep of Gr. II served as infected untreated controls.

Faecal examination by hatching and sieving method was carried out at fortnightly intervals till end of the experiment (day 60). Haematological examination for determination of haemoglobin (Hb g%), packed cell volume (PCV %) and total erythrocytic counts (TEC millions/cmm) was made on day 0, 30 and 60 post treatment (PT). All the animals were sacrificed on day 60 PT and *S. indicum* worms, pieces of liver, lungs, duodenum and caecum were collected for further examination.

3.2.3 Faecal egg counts

Faecal samples from sheep of both the groups were collected at fortnightly intervals. They were processed for egg counts as per method described earlier.

3.2.4 Haematological examination

Blood samples were collected directly from jugular vein in a sterilized

glass vial containing ethylene diamine tetra acetic acid (EDTA 1 mg/ml) as anticoagulant.

Haemoglobin (Hb g%), packed cell volume (PCV %) and total erythrocytic counts (TEC millions/cmm) were determined as per method of Jain (1986).

3.2.5 Worm recovery

Worms were recovered as per method described by Agrawal (1998). Male and female worms were counted separately for each killed sheep.

3.2.6 Tissue egg counts

One gram of tissue sample each from liver (central portion), duodenum (50 cm caudal of pylorus), caecum (central part) and lungs (central part) were collected separately in glass vials containing 15 ml of 3% potassium hydroxide (KOH). The tissues were allowed to digest for 18 hr at 25°C and the sediment was examined under stereoscope (50X) for quantitative tissue egg counts. For examination, three aliquots of 1 ml each from the sediment were examined for tissue egg counts which were multiplied by 5 to obtain EPG counts in different tissues.

3.2.7 Per cent efficacy

Per cent efficacy was estimated by comparing the number of worms recovered from treatment group with that of control.

$$\text{Per cent efficacy} = \frac{\text{Mean worm burden in sheep of infected untreated control group} - \text{Mean worm burden in sheep of infected treated group}}{\text{Mean worm burden in sheep of infected untreated control group}} \times 100$$

3.2.8 Statistical analysis

The data obtained from incidence studies was analysed by using analysis of variance (Steel and Torrie, 1960). The data for chemotherapeutic trial against natural infection of *S. indicum* was analysed by using Student's 't' test (Steel and Torrie, 1960).

4. RESULTS

4.1 Incidence

4.1.1 Field studies

Monthly incidence of *S. indicum* in sheep of Eastern Haryana for one annual cycle (August, 1999 to July, 2000) has been shown in Tab. 1 and Fig.I.

Monthly incidence data revealed that *S. indicum* infection was high from August to December with hatching (28-42%) and sieving (16-24%) methods. The incidence of this infection was low from January to April (4-8% ; 8-14%) with both the methods of detection. Thereafter a steady increase was seen in the incidence of *S. indicum* till August (42%) and then it started declining.

A distinct seasonality has been observed in the incidence pattern of *S. indicum* during the period of study (Tab.2). The comparison among seasons showed that incidence (%) was significantly ($P<0.05$) high during rainy (31.00±5.50) and post-rainy (37.00±1.00) season in relation to the summer(13.33±2.90%) and winter(16.67±5.69) season.

Table 1. Incidence pattern of *Schistosoma indicum* in 50 sheep examined monthly in Karnal district (Eastern Haryana)

Month and year	*Schistosoma indicum* eggs/miracidia with			
	Sieving method		Hatching method	
	Number Positive	Per cent positive	Number positive	Per cent positive
Aug., 1999	12	24	21	42
Sep., 1999	10	20	19	38
Oct., 1999	10	20	19	38
Nov., 1999	8	16	18	36
Dec., 1999	8	16	14	28
Jan., 2000	4	8	6	12
Feb. 2000	3	6	5	10
Mar., 2000	2	4	4	8
Apr., 2000	4	8	7	14
May, 2000	5	10	9	18
Jun., 2000	6	12	9	18
Jul., 2000	7	14	13	26

Table 2. Seasonal incidence of *Schistosoma indicum* with hatching technique in sheep of Karnal district (Eastern Haryana)

Season	Month and year	Number examined	Number positive	Per cent positive	Mean ± SE (% +ve)
Summer	Mar., 2000	50	4	8	$13.33^{b}\pm2.90$
	Apr., 2000	50	7	14	
	May, 2000	50	9	18	
Rainy	Jun., 2000	50	9	18	$31.00^{a}\pm5.50$
	Jul., 2000	50	13	26	
	Aug., 1999	50	21	42	
	Sep., 1999	50	19	38	
Post-rainy	Oct., 1999	50	19	38	$37^{a}\pm1.00$
	Nov., 1999	50	18	36	
Winter	Dec., 1999	50	14	28	$16.67^{b}\pm5.69$
	Jan., 2000	50	6	12	
	Feb., 2000	50	5	10	

Mean values with different superscripts (a, b) differ significantly at $P<0.05$

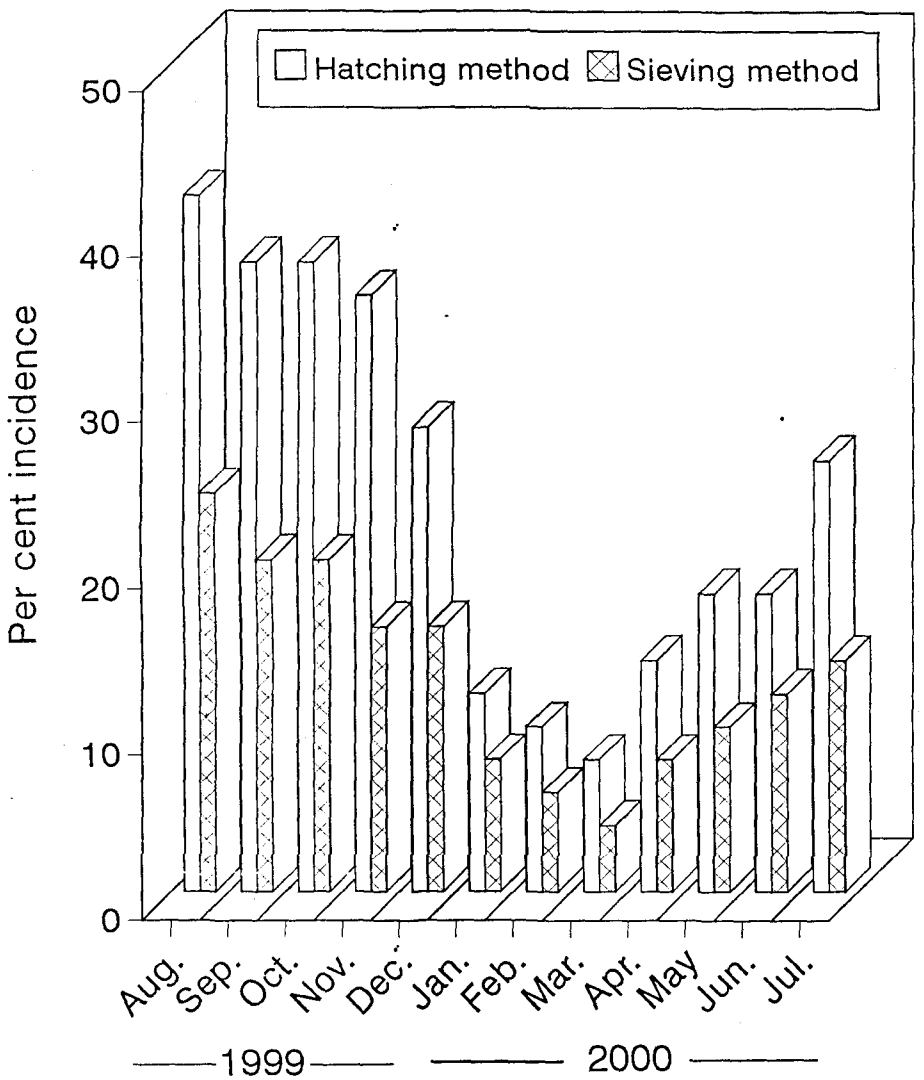


Fig. I. Incidence pattern of *Schistosoma indicum* in 50 sheep examined monthly in Karnal district (Eastern Haryana)

4.1.2 Slaughter-house data

The examination of liver pieces and faecal samples of 232 sheep, of either sex, breed and age slaughtered at Municipal Committee slaughter-house, Karnal from December, 1999 to July, 2000 have been presented in Tab.3.

In all 28 (12.08%) sheep were found positive by liver press method. The hatching and sieving methods could detect only 20 (8.62%) and 15 (6.46%) sheep positive for *S. indicum* miracidia and eggs, respectively.

Month-wise incidence showed that *S. indicum* infection was low from December to April with liver press (3.84 to7.69%), hatching(0 to 3.84%) and sieving(0 to 3.84%) methods. From May onwards, *S. indicum* infection registered an upward trend till the last month of observation.

Sex-and age-wise incidence of *S. indicum* in killed sheep have been shown in Tab.4. From the analysis of data it is evident that adults ($\geq$ 6 months) are more infected than the lambs (<6 months). Overall incidence further revealed that females (16.5%) were more infected with *S. indicum* than males (2.7%).

4.2 Chemotherapeutic trial

4.2.1 Effect on faecal egg counts

The mean faecal egg counts in sheep naturally infected with *S. indicum* and treated with praziquantel have been presented in Tab.5 and Fig.II.

Within the group, the treated sheep had highly significant ($P<0.01$) decrease in faecal egg counts on day 15 PT. Thereafter the sheep of this

Table 3. Incidence of *Schistosoma indicum* by various methods in sheep killed at Municipal slaughter house, Karnal (Eastern Haryana)

Month and year	Number Examined	*S. indicum* eggs/miracidia with					
		Liver press method		Hatching method		Sieving method	
		Number Positive	Per cent positive	Number positive	Per cent positive	Number positive	Per cent positive
Dec., 1999	26	1	3.84	0	0.00	0	0.00
Jan., 2000	27	1	3.70	0	0.00	0	0.00
Feb., 2000	30	0	0.00	0	0.00	0	0.00
Mar., 2000	26	2	7.69	1	3.84	1	3.84
Apr., 2000	26	2	7.69	1	3.84	1	3.84
May, 2000	33	5	15.15	4	12.12	3	9.09
Jun., 2000	31	8	25.80	6	19.35	5	16.12
Jul., 2000	33	9	27.27	8	24.24	5	15.15
Total	232	28	12.08	20	8.62	15	6.46

Table 4. Sex- and age-wise incidence of *Schistosoma indicum* in sheep slaughtered at Karnal from December, 1999 to July, 2000

Age (Month)	Number Examined	Number positive	Per cent positive	Sex-wise frequency					
				Male			Female		
				Number Examined	Number positive	Per cent positive	Number examined	Number positive	Per cent positive
Lamb (<6 months)	38	2	5.26	16	0	0.0	22	2	9.09
Adults (≥6 months)	194	26	13.40	58	2	3.4	136	24	17.6
Overall	232	28	12.06	74	2	2.7	158	26	16.5

Table 5. Faecal egg counts in sheep naturally infected with *Schistosoma indicum* and treated with praziquantel

Days post treatment	Post treatment *Schistosoma indicum* eggs in												Per cent reduction
	Infected treated* (Gr.I)						Infected control (Gr.II)						
	1	2	3	4	5	Mean ± S.E.	6	7	8	9	10	Mean ± S.E.	
0	50	80	30	30	90	56^{ax}±12.52	50	30	80	40	50	50^{ax}±8.37	0.0
15	10	10	0	0	10	6^{cy}±2.45	50	40	80	40	50	52^{ax}±7.35	87.5
30	0	0	0	0	0	0^{cy}	50	50	80	50	60	58^{ax}±5.83	100.0
45	0	0	0	0	0	0^{cy}	50	50	90	60	60	62^{ax}±7.35	100.0
60	0	0	0	0	0	0^{cy}	60	70	100	90	60	76^{ax}±8.12	100.0

*Sheep treated with praziquantel @ 25 mg/kg body weight orally on day 0.
Values in rows (x, y) and columns (a, b) with dissimilar superscripts differ significantly at $P<0.01$.

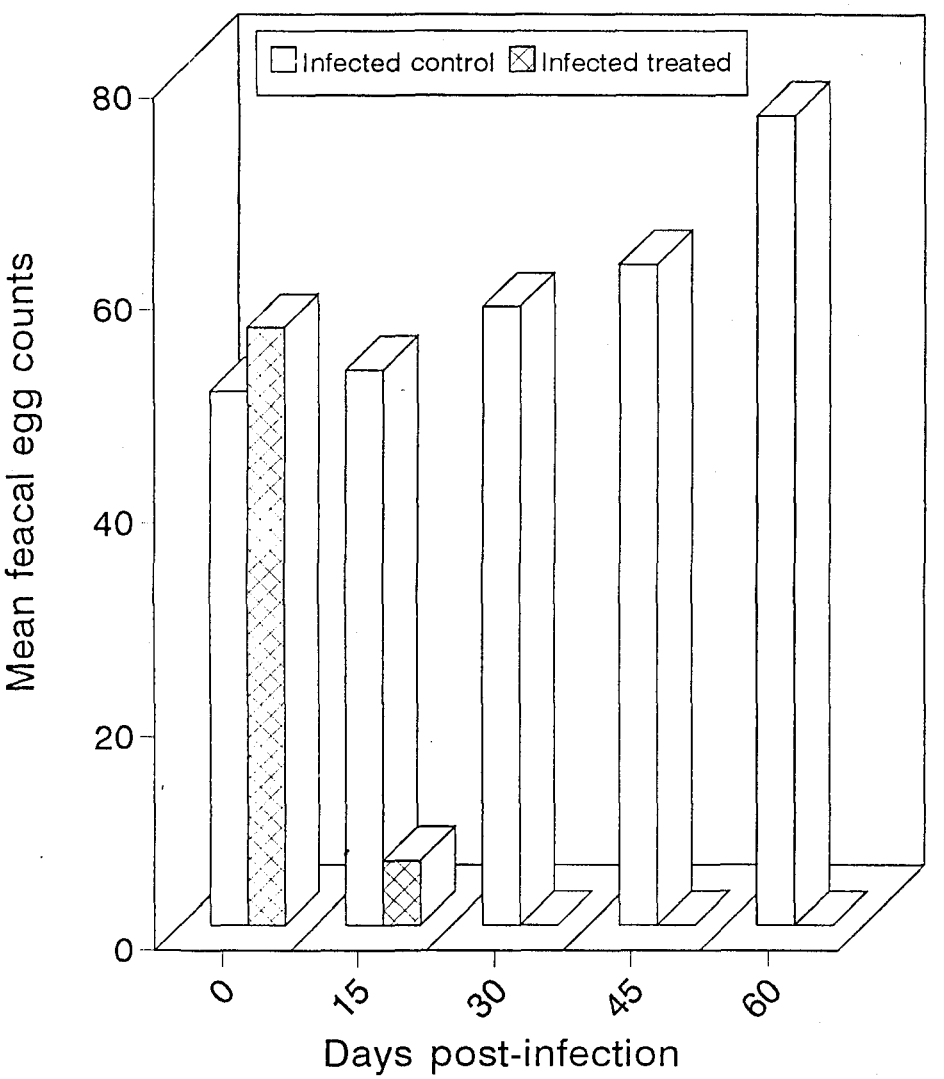


Fig. II. Faecal egg counts in sheep naturally infected with *Schistosoma indicum* and treated with praziquantel

group did not show any egg till day 60 PT. The animals of infected untreated control group did not reveal any significant difference in egg counts till the day of last observation (day 60 PT).

The comparison among groups showed highly significant ($P<0.01$) reduction in egg counts of treated animals from day 15 PT (87.5%) to day 60 PT (100%).

4.2.2 Haematology

Mean haemoglobin (Hb g%) values in sheep of both the groups (Gr I-Infected treated, Gr II-Infected untreated control) have been depicted in Tab.6. The Hb (g%) values increased significantly ($P<0.05$) in treated sheep from day 0 (7.30±0.25) PT to day 60 (9.25±0.38)PT. In the control group Hb values fell non-significantly from day 0 (7.45±0.82) to day 30 (7.00±0.42) PT. However, on day 60 (5.98±0.68) PT, the fall in Hb values was significant ($P<0.05$). Between groups, the sheep of infected treated and infected untreated control group had significant ($P<0.05$) difference on day 60 PT.

Within the groups PCV (%) values increased significantly ($P<0.05$) in the treated sheep (Gr. I) on day 30 and on day 60 PT whereas these values decreased significantly ($P<0.05$) in infected untreated control (Gr. II) animals on day 30(27±0.70) and on day 60 (24.60±0.50) PT. PCV in infected treated and infected untreated control sheep revealed significant ($P<0.05$) difference on day 30 and on day 60 PT (Tab.7).

Table 6. Post treatment haemoglobin (Hb g%) in sheep naturally infected with *Schistosoma indicum*

Days post treatment	Infected treated* (Gr. I)						Infected control (Gr. II)					
	1	2	3	4	5	Mean ±SE	6	7	8	9	10	Mean ± SE
0	7.08	8.01	7.38	7.01	7.05	7.30^{ax}±0.25	7.48	8.28	6.20	7.91	7.62	7.45^{ax}±0.82
30	9.12	9.18	9.22	8.98	8.58	8.97^{ay}±0.49	6.99	8.08	6.11	6.86	6.98	7.00^{bx}±0.42
60	10.01	9.11	9.21	9.09	9.05	9.25^{ay}±0.38	6.01	6.99	5.08	5.90	5.86	5.98^{by}±0.68

*Sheep of Gr. I were treated with praziquantel @ 25 mg/kg body weight orally on day 0.
Values with superscripts in rows (x, y) and in columns (a, b) differ significantly at $P<0.05$

Table 7. Post treatment packed cell volume (PCV %) in sheep naturally infected with *Schistosoma indicum*

Days post treatment	Infected treated* (Gr. I)						Infected control (Gr. II)					
	1	2	3	4	5	Mean ±SE	6	7	8	9	10	Mean ± SE
0	31	30	38	27	28	28.80^{ax}±0.73	30	28	27	32	30	29.40^{ax}±0.87
30	32	33	31	30	30	31.20^{ay}±0.58	27	26	25	29	28	27^{bz}±0.70
60	34	33	32	30	32	32.20^{ay}±0.66	26	25	23	25	22	24.60^{bz*}±0.50

*Sheep of Gr. I were treated with praziquantel @ 25 mg/kg body weight orally on day 0.
Values in rows (x, y, z, z*) and columns (a, b) with dissimilar superscripts differ significantly (b, y, z = $P<0.05$; z* = $P<0.01$).

Individual and mean TEC (million/cmm) values in both the groups of sheep are shown in Tab.8. Within group, infected treated sheep had significant($P<0.05$) increase in TEC on day 30 and on day 60 (7.67±0.15) PT. The infected untreated control sheep had significant ($P<0.05$) fall in TEC values on day 30 (6.19±0.08) and on day 60 (6.00±0.05) PT. Between the groups, the TEC differed signicantly ($P<0.05$) on day 30 and on day 60 PT.

The results of Tab.9 showed that the values of mean corpuscular volume (MCV cu μ), mean corpuscular haemoglobin (MCH μ μg) and mean corpuscular haemoglobin concentration (MCHC %) varied between 41.68±1.17 to 43.83±3.74, 10.80±0.33 to 12.12±0.26 and 23.99±1.30 to 28.95±0.44, respectively in sheep of infected treated group.The comparison of these values with the sheep of infected untreated control group revealed non significant difference in MCV but MCH and MCHC differed significantly ($P<0.05$) on day 60 PT.

4.2.3 Worm recovery

Mean number of male, female and total worms recovered by perfusion and tissue chopping on day 60 PT in each group have been presented in Table 10. In all sheep more males than females were found. Total number of worms recovered from Gr. I was reduced by 98.9 per cent when compared with Gr. II and this reduction was highly significant ($P<0.01$). Further, statistical analysis revealed that there was no significant difference in male (99.39%) and female worm (98.42%) reduction.

Table 8. Post treatment total erythrocytic counts (TEC millions/cmm) in sheep naturally infected with *Schistosoma indicum*

Days post treatment	Infected treated* (Gr. I)						Infected control (Gr. II)					
	1	2	3	4	5	Mean ±SE	6	7	8	9	10	Mean ± SE
0	6.98	7.01	6.28	6.88	6.70	6.77^{ax}±0.13	6.49	6.92	6.65	6.85	6.95	6.77^{ax}±0.08
30	7.70	7.50	7.10	8.02	7.18	7.50^{ay}±0.17	6.35	6.01	6.28	5.98	6.32	6.19^{by}±0.08
60	7.92	7.80	7.32	8.01	7.29	7.67^{ay}±0.15	6.18	5.88	6.01	5.91	6.02	6.00^{by}±0.05

*Sheep of Gr. I were treated with praziquantel @ 25 mg/kg body weight orally on day 0.
Values in rows (x, y) and columns (a, b) with dissimilar superscripts differ significantly at $P<0.05$

Table 9. Mean ±SE values of mean corpuscular volume (MCV cu μ), mean corpuscular haemoglobin, (MCH μ μg) and mean corpuscular haemoglobin concentration (MCHC %) in sheep

Days post Treatment	MCV		MCH		MCHC	
	Gr. I	Gr. II	Gr. I	Gr. II	Gr. I	Gr. II
0	43.83±3.74	43.43±1.15	10.80±0.33	11.06±0.46	23.99±1.30	25.5±1.09
30	41.68±1.17	43.67±1.41	12.03±0.29	11.33±0.60	28.90±0.40	26.00±1.34
60	42.05±1.18	40.34±1.23	12.12*±0.26	9.95±0.55	28.95*±0.44	24.59±1.06

Gr.I = Infected treated;

Gr. II = Infected untreated control.

*Significant ($P<0.05$).

Table 10. Post-mortem worm recovery of adult *Schistosoma indicum* from naturally infected sheep after treatment with praziquantel

Worm recovery	Worm recovery of adult *Schistosoma indicum* eggs/miracidia in different groups on day 60 PT												Per cent reduction
	Infected treated* (Gr. I)						Infected control (Gr. II)						
	1	2	3	4	5	Mean ±SE	6	7	8	9	10	Mean ± SE	
Male	0	0	0	2	3	1^{ax}±0.63	173	132	203	205	115	165.6^{cx}±27.33	99.39
Female	2	0	0	3	5	2^{ax}±0.95	90	156	194	127	69	127.2^{cx}±22.49	98.42
Total	2	0	0	5	8	3^{ax}±1.55	263	288	397	332	184	272.8^{cy}±35.36	98.9

*Sheep of Gr. I were treated with praziquantel @ 25 mg/kg body weight orally on day 0.
Values in rows (x, y) and columns (a, c) with dissimilar superscripts have highly significant difference at $P<0.01$.
PT = Post treatment.

4.2.4 Tissue egg counts

Mean tissue egg counts per gram of different tissues viz. liver, duodenum, caecum and lungs in sheep naturally infected with *S. indicum* and treated with praziquantel have been shown in Table 11 and Fig. III. Tissue egg counts per gram of liver decreased significantly ($P<0.05$) in infected treated sheep in comparison to infected untreated group. In infected untreated control group highest tissue egg counts were found in caecum (768±44.00) followed by liver(663±54.93),duodenum (528±20.97) and lungs (12±12.00). The comparison in between the groups showed highly significant ($P<0.01$) reduction in tissue egg counts of treated sheep in caecum (96.8%), duodenum (96.02%) and lungs (100%). In contrast to these observations, reduction was less pronounced in liver (48.66%).

Table 11. Post-mortem tissue egg counts of *Schistosoma indicum* in naturally infected sheep after treatment with praziquantel

Organ	Tissue egg counts of *S. indicum* on day 60 PT												Per cent reduction
	Infected treated* (Gr. I)						Infected control (Gr. II)						
	1	2	3	4	5	Mean ±SE	6	7	8	9	10	Mean ± SE	
Liver	450	330	285	210	420	339^{a}±44.00	570	645	810	765	525	663^{b}±54.93	48.86
Lung	0	0	0	0	0	0^{a}	0	0	60	0	0	12^{d}±12.00	100.00
Duodenum	15	30	30	15	15	21^{a}±3.68	450	525	570	540	555	528^{c}±20.97	96.02
Caecum	15	30	30	15	30	24^{a}±3.68	690	720	885	810	735	768^{b}±35.40	96.80

*Sheep of Gr. I were treated with praziquantel @ 25 mg/kg body weight orally on day 0.
Values in columns (a, b, c, d) with dissimilar superscripts are significantly different.
(b = $P<0.05$, c = $P<0.01$, d = $P<0.001$).
PT = Post-treatment.

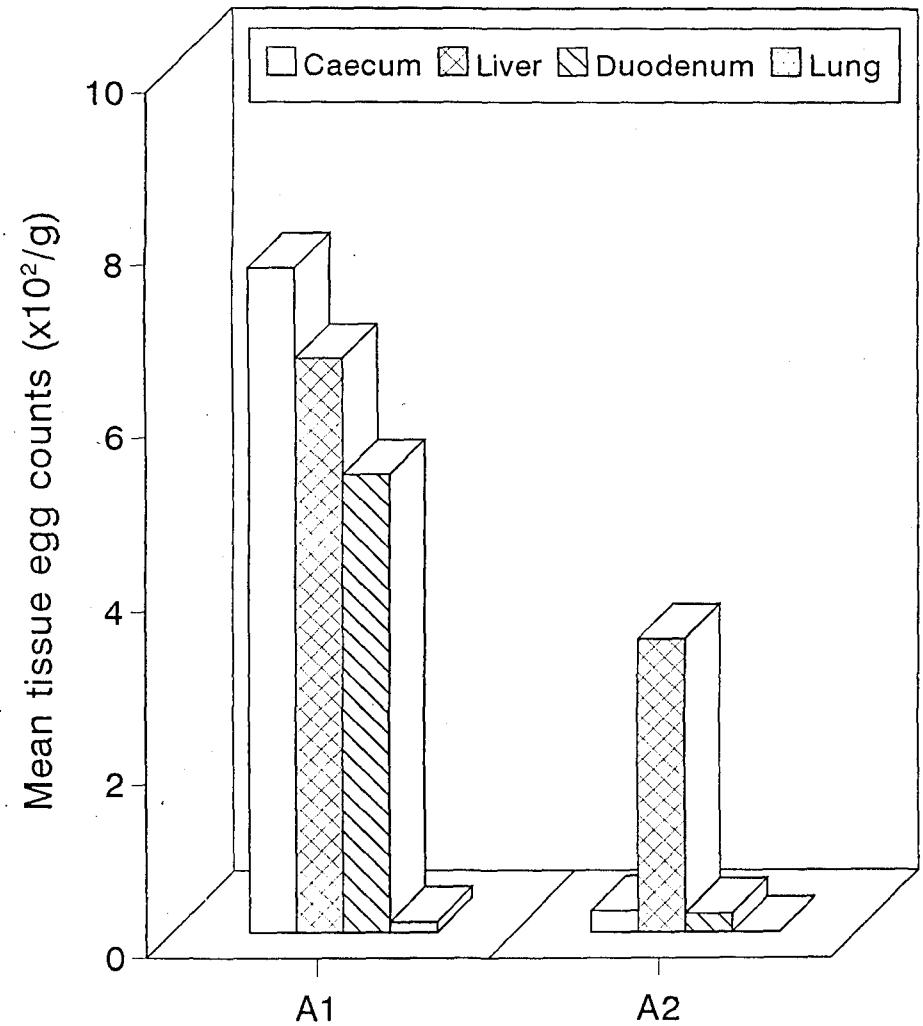
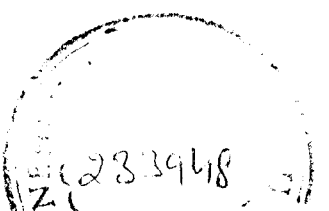


Fig. III. Post-mortem tissue egg counts of *Schistosoma indicum* in naturally infected sheep after treatment with praziquantel

A1 - Infected control
A2 - Infected treated

5. DISCUSSION

Schistosoma indicum, the cause of schistosomosis, in sheep of Karnal district (Eastern Haryana) has been observed with high incidence from August to December and low from January to April. The observed incidence may be explained on the basis of the incidence pattern of *Indoplanorbis exustus* which acts as an intermediate host of *S. indicum* (Srivastava and Dutt, 1962). This aquatic snail is prevalent in abundance in Eastern Haryana during July to October (Chaudhri *et al.*, 1982a, b; Gupta *et al.*, 1987). The limited studies on incidence of *S. indicum* cercariae in *I. exustus* in this area revealed prevalence of furcocercous cercariae (mammalian) from August to January (Gupta *et al.*, 1987). Agrawal (1981) also noticed in Jabalpur (MP), an increase in snail population from July to December with schistosome cercariae appearing from August to February. Thus, it can be assumed that animals get infection from July onwards. Since prepatent period of *S. indicum* in lambs is 52 days (Srivastava and Dutt, 1951), therefore, it is expected that susceptible animals after getting infection will start shedding eggs towards the end of August and thereafter infection will increase.



However, the observed pattern in present study with high incidence from August to December and low incidence from January to April indicate the involvement of factors other than the mere acquisition of infection through schistosome cercariae. Such factors may include the longer survival period (972 days) of *S. indicum* in sheep (Srivastava and Dutt, 1962).

A distinct seasonality in the incidence pattern of *S. indicum* has been observed during present study . Incidence was significantly higher in rainy and post-rainy seasons than in winter and summer seasons. This could be attributed to ecological behaviour of snail intermediate host and agroclimatic conditions of the area (Chaudhri *et al.*, 1993). Somewhat similar studies were also made by Mohan Rao and Choudary (1984) who also recorded highest incidence of *S. indicum* infection in sheep of Andhra Pradesh during August-March and not even a single case of *S. indicum* in the remaining months. Rajkhowa *et al.* (1992a) also observed that incidence of *S. indicum* in cattle gradually rises from August (35.29%) to February (42.85%) reaching a peak in December (61.4%).

Presently hatching method proved more sensitive in detecting *S. indicum* infection in sheep than sieving method of faecal examination as reported earlier by Banerjee and Agrawal (1992) in cattle and buffaloes.This may be due to fact that much larger faecal samples can be used in hatching technique which increases sensitivity to detect even light infection.

The liver examination of 232 sheep at Municipal Committee slaughter house, Karnal from December, 1999 to July, 2000 detected *S. indicum*

infection in 12.08 per cent animals. The previous studies on presence of *S. indicum* infection in liver tissues of slaughtered sheep by various workers (Rao, 1947; Arora and Iyer, 1968; Nara and Nayak, 1972; Mohan Rao and Choudary, 1984; Banerjee and Agrawal, 1989) in different states revealed a variation ranging in between 0.75 to 54.0 per cent. The difference in per cent infection of *S. indicum* to that of the present study may be due to focal nature of this infection and also local agro climatic conditions of a particular area.

The present observations revealed liver press method to be the most sensitive in comparison to hatching and sieving methods. Banerjee and Agrawal (1989) also observed liver press method to detect *S. indicum* infection in 54 per cent cattle in comparison to 44 per cent and 20 per cent by hatching and sieving methods, respectively. In another study, Agrawal and Panesar (1987) found sensitivity of liver press method more than hatching technique in sheep. Rajkhowa *et al.* (1992a) from Assam also observed liver press method more sensitive in detecting *S. indicum* infection in cattle, buffaloes and goats than acid ether technique of faecal examination. Thus, it is evident that liver press method is the most sensitive technique to detect *S. indicum* infection in liver tissues of slaughtered animals.

Sieving method proved to be the least sensitive which was able to detect *S. indicum* infection in 15(6.46%) sheep out of 232 examined.While comparing different diagnostic methods for schistosomosis Banerjee and Agrawal (1989) also reported sieving method to be least sensitive where it

detected only 20% cattle and 13.3% buffaloes positive for *S. indicum* as compared to 44% cattle and 40% buffaloes by liver press method. Sensitivity of sieving method may be low due to less quantity of faecal sample examined. So a sensitive diagnostic method is needed to study incidence rate fairly.

More infection of *S. indicum* in adults (≥6 months) than in lambs (<6 months) is similar to earlier reports of Islam (1975) who observed higher prevalence and intensity of infection in mature cattle and goats than in younger animals. The higher prevalence of *S. indicum* infection in adults than in younger animals could be due to absence of effective age related resistance against schistosomosis in sheep (Majid *et al.*, 1983) and also due to more exposure of adults to infection than younger ones. Further higher infection of *S. indicum* in females than in males observed during present study may be due to the involvement of various stress factors including parturition, lactation, pregnancy and nutrition etc.in the former.

Praziquantel [2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-2H-pyrazino-(2,1-a) isoquinoline-4-one], synthesized by J. Seubert *et al.* (1977), is a crystalline and almost colourless substance which was found highly effective for some cestodes and trematodes including schistosomes (Davis *et al.*, 1979). Praziquantel causes rapid contraction of schistosome musculature, probably due to changes in permeability of their cell membrane (Pax *et al.*, 1978). Secondary effects are perhaps the reduced endogenous glycogen, a decrease in alanine and an increase in lactate release (Groll, 1984).

This drug is almost completely absorbed from gastrointestinal tract and effective serum concentration reaches within 30 min to1 hr (Steiner *et al.*, 1976). It has been reported to be eliminated via its metabolites through urine and faeces within 4 days (Leopold *et al.*, 1978). Praziquantel has been successfully used in animals against *S. bovis* (Bushara *et al.*, 1982, 1983; Morkovics *et al.*, 1985; Johansen *et al.*, 1996), *S. spindale* (Upatoom *et al.*, 1988) and *S. nasale* (Anandan and Raja, 1987).

Presently, praziquantel @ 25 mg/kg body weight was found highly effective against natural infection of *S. indicum* in sheep. This resulted in highly significant ($P<0.01$) reduction in faecal egg counts on day 15 (87.5%) PT. Faecal egg counts reduced to zero from day 30 PT onwards in the treated sheep. A complete reduction in faecal egg counts by day 60 PT was also reported by Upatoom *et al.* (1988) in calves naturally infected with *S. spindale* when praziquantel was given @ 25 mg/kg body weight orally or intramuscularly. Bushara *et al.* (1982) also recorded highly significant reduction in faecal egg counts of calves experimentally infected with *S. bovis* on week 1 and 3 PT with praziquantel @ 20 mg/kg body weight given twice on week 9 and 14 PI. In a similar study on goats experimentally infected with *S. bovis* and treated with praziquantel @ 60 mg/kg body weight orally on week 13 PI, Johansen *et al.* (1996) also observed a reduction in faecal egg counts by 84.1 per cent and 98.3 per cent on week 1 and 3 PT, respectively. Thus it can be deduced that praziquantel is highly effective in

reducing the faecal egg counts of *Schistosoma spp.* in treated sheep, calves and goats.

The infected treated sheep showed significant increase in haemoglobin, packed cell volume and total erythrocytic counts on day 30 and 60 PT indicating improvement in anaemic status of treated animals. These haematological values in praziquantel treated sheep have not been studied earlier. In contrast to these observations, the sheep of infected untreated control group exhibited significant reduction in haematological parameters from day 0 to day 60 PT indicating development of anaemia with passage of time. Previously, Srivastava *et al.* (1964) also recorded significant reduction in Hb, PCV and TEC in lambs experimentally infected with 7000-15000 cercariae of *S. indicum* orally in 9-26 daily doses of 200-1300 cercariae per dose. When data for haematological parameters was interpreted (Coles,1967), the infected sheep showed normocytic normochromic anaemia. Rajkhowa *et al.* (1992b) from Assam also reported normocytic normochromic anaemia in calves infected intranasaly with 8800-12200 *S. indicum* cercariae.

Anaemia in schistosomosis may be due to blood loss in faeces, reduced cell survival and expansion of plasma volumes leading to haemodilution (Holmes *et al.*, 1977). Dyshaemopoisis may be another attributing factor in development of anaemia in this disease (Preston and Dargie, 1974). From the present observations, it is abundantly clear that praziquantel is effective in reducing anaemia in treated sheep.

Mean worm burden in treated sheep sacrificed on day 60 PT reduced to 98.9 per cent. Bushara *et al.* (1982) also found praziquantel @ 20 mg/kg body weight, administered orally on two occasions i.e. week 9 and 14 PI, to be highly (98.9%) effective in reducing the worm burden in calves experimentally infected with *S. bovis*. Johansen *et al.* (1996) also recorded 99.4% worm reduction in goats experimentally infected with *S. bovis* and treated with praziquantel @ 60 mg/kg body weight orally. Thus it is evident that praziquantel is equally effective against *S. indicum* and *S. bovis* in sheep, goats and calves.

This drug was also found highly effective in reducing tissue egg counts at autopsy. Reduction was highly significant in caecum (96.80%) and duodenum (96.02%) but less pronounced in liver (48.86%). While examining the different tissues of killed goats experimentally infected with *S. bovis* and treated with praziquantel (@ 60 mg/kg body weight orally), Johansen *et al.* (1996) also recorded a significant reduction in tissue egg counts of small intestine, caecum and colon with a non significant reduction in tissue egg counts of liver. The lower reduction in liver tissue egg counts may be due to anatomical and physiological conditions prevailing in this organ (Johansen *et al.*, 1996). However, the significant reduction of *S. indicum* eggs in intestinal tissues further confirms the high efficacy of this drug in sheep.

6. SUMMARY AND CONCLUSIONS

The incidence studies on *S. indicum* in sheep of district Karnal (Eastern Haryana) were carried out for one annual cycle (August, 1999 to July, 2000). Rectal faecal samples of 50 sheep from two selected flocks in villages nearby Parasitological Research Centre, CCS HAU Regional Research Centre, Karnal, were collected monthly and examined by hatching technique and sieving method. A high incidence of this infection was recorded from August to December. Seasonal pattern revealed significantly higher incidence of *S. indicum* infection in sheep during rainy and post-rainy seasons and low during summer and winter seasons.

Examination of liver pieces and faecal samples of 232 sheep slaughtered at Municipal Committee slaughter-house, Karnal, revealed *S. indicum* infection in 12.08 per cent livers. Comparison among different diagnostic methods employed during the study showed that liver press method was the most sensitive followed by hatching and sieving methods.

Age-wise analysis of data revealed adults sheep ($\geq$6 months) to be more infected with *S. indicum* than lambs (<6 months). Sex-wise incidence of this infection was higher in females than in males.

In a subsequent experiment, praziquantel was evaluated against natural *S. indicum* infection in sheep under controlled conditions. The sheep were divided into two equal groups (Gr. I, infected treated, Gr. II, infected untreated control). Sheep of Gr. I were treated with praziquantel @ 25 mg/ kg body weight orally. The efficacy of drug was evaluated by comparing EPG at different intervals, haematological parameters, total worm recovery and tissue egg counts between infected treated and infected untreated control animals. The animals were sacrificed on day 60 PT and flukes were recovered by perfusion technique.

Praziquantel reduced faecal egg counts by 87.5 per cent on day 15 and 100 per cent on day 30, 45 and 60 PT. Significant increase in haematological values in treated sheep from day 0 PT to day 30 and 60 PT indicated high efficacy of drug in terms of improvement in anaemic status of the animal.

Significant reduction in total worm burden of praziquantel treated sheep indicates that drug is highly effective (98.9%) against *S. indicum*. Tissue egg counts were reduced significantly in caecum and duodenum but not in the liver.

All these observations indicate that praziquantel can be successfully used to control schistosomosis in sheep in this region of Haryana. High efficacy (98.9%) of praziquantel against *S. indicum* in sheep is reported for the first time.

Conclusions

* Schistosomosis caused by *S. indicum*, has a definite pattern with high incidence from August to December and low incidence from January to July in sheep of Karnal district (Eastern Haryana).
* Hatching method is recommended as a valuable diagnostic tool for *S. indicum* infection in domestic animals.
* Liver press method has been found to be most sensitive to detect *S. indicum* infection in slaughtered sheep.
* Adults sheep ($\geq$6 months) are more infected with *S. indicum* than lambs (< 6 months).
* Praziquantel @ 25 mg/kg body weight orally is highly effective in reducing faecal egg counts and worm counts to 100 per cent and 98.9 per cent, respectively. Hence it can be used for control of hepato-intestinal schistosomosis in sheep.

LITERATURE CITED

Agrawal, M. C. 1981. Some observations on schistosomiasis in Jabalpur area. *Livestk. Advis.* **53**:505-58.

Agrawal, M.C. 1998. New technique for infection and recovery of schistosomes from animals. *Indian J. Anim. Sci.* 68(6):521-523

Agrawal, M.C. and Panesar, N. 1987. Evaluation of diagnostic techniques for natural porcine schistosomiasis. *Indian Vet. J.* **64**: 918-920.

Agrawal, M.C. and Sahasrabudhe, V.K. 1982. Evaluation of routine diagnostic methods for detecting hepato-intestinal schistosomiasis in cattle and goats. *Indian J. Parasitol.* **6**: 319-320.

Anandan, R. and Raja, E.E. 1987. Preliminary trials with praziquantel in *Schistosoma nasale* infection in sheep and goat. *Indian Vet. J.* **64**: 108-110.

Aradaib, I.E.; Abbas, B.; Bushara, H.O. and Taylor, M.G. 1993. Evaluation of *Schistosoma bovis* adult worm extract for vaccination of calves. *Prev. Vet. Med.* **16**: 776-784.

Aradaib, I.E.; Omer, O.H.; Abbas, B.B.; Bushara, H.O.; Elmalik, K.H.; Saad, A.M.; Osburn, B.I. and Taylor, M.G. 1995. *Schistosoma bovis* whole egg antigen did not protect zebu calves against experimental schistosomiasis. *Prev. Vet. Med.* **21**: 339-345.

Arora, R.G. and Iyer, P.K.R. 1968. Observations on the pathology of ovine and caprine livers infected with *Schistosoma indicum* (Montagomery, 1906). *Indian J. Anim. Hlth.* 7(1): 67-74.

Banerjee, P.S. and Agrawal, M.C. 1989. Comparative efficacy of faecal and liver examination in determining prevalence of bovine schistosomiasis. *J. Vet. Parasitol.* **3**(2): 157-158.

Banerjee, P.S. and Agrawal, M.C. 1992. Epizootiological studies in bovines on fluke infections with special reference to schistosomiasis. *Indian Vet. J.* **69**: 215-220.

Bhalerao, G.D. 1932. On the identity of schistosomes found in cases of bovine nasal granuloma and some observations on a few other members of the schistosomatidae. *Indian J. Vet. Sci. Anim. Husb.* **2**: 338-356.

Bhaskararao, P.; Karkhani, R.S.; Sivasankar, V. and Gupta, T.V.L.S. 1984. An endemic of *Schistosoma indicum* in Andhra Pradesh. *Livestk. Advis.* **9**: 61-62.

Bhatia, B.B. 1960. A note on liver affections with 3 species of flukes parasitizing Indian sheep. *Indian J. Helminth.* **12**(2): 74-79.

Bushara, H.O.; Bashir, M.E.N.; Malik, K.H.E.; Mukhtar, M.M.; Trottein, F.; Capron, A. and Taylor, M.G. 1993. Suppression of *Schistosoma bovis* eggs in cattle by vaccination with either glutathion S-transferase or keyhole limpet haemocyanin. *Parasite Immunol.* **15**: 383-390.

Bushara, H.O.; Hussein, M.F.; Majid, M.A. and Taylor, M.G. 1982. Effects of praziquantel and metrifonate on *Schistosoma bovis* infections in Sudanese cattle. *Res. Vet. Sci.* **33**: 125-126.

Bushara, H.O.; Hussein, M.F.; Saad, A.M.; Taylor, M.G.; Dargie, J.D.; Marshall, T.D. de C. and Nelson, G.S. 1978. Immunisation of calves against *Schistosoma bovis* using irradiated cercariae or schistosomula of *S. bovis*. *Parasitology.* 77: 303-311.

Bushara, H.O.; Majid, B.Y.A.; Majid, A.A.; Khitma, I.; Gameel. A.A.; Karib, E.A.; Hussein, M.F. and Taylor, M.G. 1983. The effects of praziquantel therapy on naturally acquired resistance to *Schistosoma bovis*. *Amer. J. Trop. Med. Hyg.* **32**: 1370-1374.

Chandler, A.C. 1926. A new schistosome infection of man, with notes on other human fluke infections in India. *Indian J. Med. Res.* **14**: 120-121.

Chaudhri, S.S.; Gupta, R.P. and Sangwan, A.K. 1993. Helminthic diseases in ruminants of Haryana and their control-A review. *Agric. Rev.* **14**(3): 121- 132.

Chaudhri, S.S.; Gupta, R.P. and Yadav, C.L. 1982a. Note on cercarial fauna of some aquatic snails of Haryana state. *Indian J. Anim. Sci.* **52**(12): 1273-1275.

Chaudhri, S.S.; Gupta, R.P. and Yadav, C.L. 1982b. A note on bionomics of some aquatic snails of Haryana state. *Haryana Vet.* **21**: 60-64.

Chaudhri, S.S.; Kumar, Sudesh and Gupta, R.P. 1994. Prevalence of *Schistosoma indicum* in Haryana and treatment trials in sheep. *Indian Vet. J.* **71**: 291-292.

Cobbold, T.S. 1882. (Cited from Baugh, S.C. 1978. A centuary of schistosomiasis in India. *Rev. Iber. Parasitol.* **38**: 435-473).

Coles, E.H. 1967. Veterinary Clinical Pathology. W.B.Saunders Company, Philadelphia and London. PP. 84-85.

Dadhich, H. and Sharma, G.D. 1996. Pulmonary schistosomiasis in goats in Rajasthan. *Indian Vet. J.* **73**: 677-678.

Dargie, J.D. 1980. Pathogenesis of *Schistosoma bovis* infection in Sudanese cattle. *Trans. Royal Soc. Trop. Med. Hyg.* **74**: 560-562.

Datta, S.C.A. 1933. *Schistosoma indicum* Montgomery (1906), as the cause of persistent debility in equines in India, with a description of the lesions. *Indian J. Vet. Sci. Anim. Husb.* **3**(1): 1-28.

Davis, A. 1993. Antischistosomal drugs and clinical practice. In: Human Schistosomiasis (P. Jordan, G. Webbe and R.F. Sturrock, eds.), PP. 367-404. Wallingford : CAB International.

Davis. A.; Biles, J.E. and Ulrich, A.M. 1979. Efficacy of praziquantel in human schistosomiasis. *Bull. WHO.* **57**: 773-779.

De Bont, J. and Vercruysee, J. 1998. Schistosomiasis in cattle. *Adv. Parasitol.* **41**: 285-363.

Dutt, S.C. and Srivastava, H.D. 1952. On the morphology and life history of a new mammalian blood fluke-*Orientobilharzia dattai* n.sp. (preliminary report). *Parasitology.* **42**: 144-150.

Dutt, S.C. and Srivastava, H.D. 1961. On the epidermal structure of the miracidia of six species of mammalian schistosomes and a new

technique of specific diagnosis of animal schistosomiasis. *Indian J. Helminth.* **13**: 100-111.

Dutt, S.C.; Chandra, R.; Muralidharam, S.R.G. and Srivastava, H.D. 1963. Chemotherapy of *Schistosoma indicum* infection in sheep. *Annals Biochem. Exp. Med.* **23**(11): 439-446.

Endrejat, E. 1964. Some observations of schistosomiasis in Assam. *Indian Vet. J.* **41**: 538.

FAO. 1999. Food and Agricultural Organisation. *Quarterly Bulletin of Statistics*. **12**(½): 83-84.

Groll, E. 1984. Praziquantel. *Adv. Pharmacol. Ther.* **20**:219-236.

Gupta, R.P.; Yadav, C.L. and Ruprah, N. S. 1987. Studies on bionomics of some aquatic snails and their cercarial fauna of Haryana state. *Indian Vet. Med. J.* **11**:77-83.

Holmes, P.H.; Maclean, J.M.; James, E.R.; Nelson, G.S. and Taylor, M.G. 1977. Some aspects of Pathogenesis of acute schistosomiasis (*S. mansoni*) in baboons. *Trans. Royal Soc. Trop. Med. Hyg.* **71**(4): 289.

Islam, K.S. 1975. Schistosomiasis in domestic ruminants in Bangladesh. *Trop. Anim. Hlth. Prodn.* 7(4): 244.

Jain, N.C. 1986.Schalm's Veterinary Haematology. 4th edn. Lea and Febiger, Philadelphia.

Jain, P.C. 1972. Observations on the pathology of ovine livers infected with helminthic parasites. *Orissa Vet. J.* 7(2-3): 61-66.

Johansen, M.V.; Monrad, J. and Christensen, N.Q. 1996. Effects of praziquantel on experimental *Schistosoma bovis* infection in goats. *Vet. Parasitol.* **62**: 83-91.

Jordan, P., Christie, J.D. and Unrau, G.O. 1980. Schistosomiasis transmission with particular reference to possible ecological and biological methods of control. *Acta Tropica.* **37**: 95-135.

Kaur, N. 1985. Comparative studies on serodiagnosis and faecal examination in experimental schistosomiasis. *Ph.D. Thesis*, Rani Durgavati Vishwa Vidyalaya, Jabalpur (Cited Banerjee and Agrawal, 1989).

Khan, F.A.; Swarankar, C.P.; Singh, D.; Jayasankar, J. and Bhagwan, P.S.K. 1999. Ovine gastrointestinal parasitism in farmers flocks under semi-arid conditions of Rajasthan. *Indian J. Small Rum.* **5**(2): 65-70.

Kumar, V. and de Burbure, G. 1986. Schistosomes of animals and man in Asia. *Helminthl. Abst. (Series A).* **55**: 469-480.

Lawrence, J.A. 1978. Bovine schistosomiasis in Southern Africa. *Helminthl. Abst.* **47**: 261-270.

Leopold, G.; Ungethum, W.; Groll, E.; Dickmann, H.W.; Nowak, H. and Wegner, D.H.E. 1978. *Eur. J. Clin. Pharmacol.* **14**: 281-291 (Cited Groll, E. 1984).

Li, Z.D.; Huang, Y.C. and Ai, K.S. 1984. Treatment of bilharziasis in cattle with praziquantel by oral administeration and omasal injection. *Chi. J. Vet. Med.* **10**(9): 16-17.

Lodha, K.R.; Raisinghani, P.M.; Sharma, G.D.; Pant, U.V.; Arya, P.L. and Vyas,U.K.1981. Note on an outbreak of pulmonary schistosomiasis in arids of Rajasthan. *Indian J. Anim. Sci.* **51**(3): 382-385.

Madsen, H. 1990. Biological methods for the control of freshwater snails. *Parasitol. Today.* **6**: 237-241.

Majid, A.A.; Bushara, H.O.; Saad, A.M.; Hussein, M.F.; Taylor, M.G.; Dargie, J.D.; Marshall, T.F. de C. and Nelson, G.S. 1980. Observations on cattle schistosomiasis in the Sudan, a study in comparative medicine. III. Field testing of an irradiated *Schistosoma bovis* vaccine. *Amer. J. Trop. Med. Hyg.* **29**: 452-455.

Majid, A.A.; Hussein, M.F. and Taylor, M.G. 1983. Age specific prevalence and intensity of *Schistosoma bovis* infection in Sudanese desert sheep in the White Nile Province. *Res. Vet. Sci.* **35**: 120-121.

Malkani, D.G. 1933. Kumri. *Indian Vet. J.* **5**: 190-192.

Markovics, A.; Perl, S.; Chaimovitz, M.; Klopfer, U. and Pipano, E. 1985. Efficacy of praziquantel in *Schistosoma bovis* infections. *Israel J. Med. Sci.* **21**: 712.

Markovics, A.; Perl, S.; Orgad, U. and Pipano, E. 1993. Outbreak of schistosomiasis (*S. bovis*) in cattle and sheep. *Israel J. Vet. Med.* **48**(3): 123-125.

Massoud, J. and Nelson, G.S. 1972. Studies on heterologous immunity in schistosomiasis. *Bull. W.H.O.* **47**: 591-600.

McCauley, E.H.; Tayeb, A. and Majid, A.A. 1983. Owner survey of schistosomiasis mortality in Sudanese cattle. *Trop. Anim. Hlth. Prodn.* **15**: 227-233.

Minnet, F.C. 1950. Mortality in sheep and goats in India. *Indian J. Vet. Sci.* **20**: 69-103.

Mohan Rao, M.R.K. and Choudary, Ch. 1984. Pathology of visceral schistosomiasis in Andhra Pradesh. *Cheiron.* **13**(6): 328-330.

Montgomery, R.E. 1906. Observations on bilharziasis among animals in India. *J. Trop. Vet. Sci.* **1**: 138-174.

Mudaliar, S.V. and Ramanujachary, G. 1945. *Schistosoma nairi* n.sp. from an elephant. *Indian Vet. J.* **22**: 1-4.

Murthy, G.N. 1950. Schistosomiasis in cattle. *Indian Vet. J.* **27**(3): 204-205.

Nara, R.R.S. and Nayak, B.C. 1972. Studies on pathology of liver in sheep and goats. 1. Schistosomiasis. *J. Rem. Vet. Corps.* **11**(1): 9-15.

Pax, R.; Bennett, J.L. and Fetterer, R. 1978. Naunyn-Schmiedebergs Arch. Exp. Path. Pharmacol. 304: 309-315 (Cited Groll, E. 1984).

Preston, J. M. and Dargie, J.D. 1974. Pathophysiology of ovine schistosomiasis. 5. Onset, development of anaemia in sheep experimentally infected with *Schistosoma mattheei*. *J. Comp. Path.* **84**(1): 73-81.

Quin Cheng Gui. 1996. Comments on enhancement of therapeutic efficacy

of praziquantel in treatment of schistosomiasis in cattle. *Chi. J. Vet. Med.* **22**(7): 16-17.

Raghwan, R.S. 1958. Nodular cirrhosis of liver in equines. *Indian Vet. J.* **35**: 387-389.

Rai, P. 1960. Studies on blood flukes of domestic animals. 1. Some observation on natural infection in equines with *Schistosoma indicum* Montgomery (1906). *Indian J. Vet. Sci. Anim. Husb.* **30**: 38-42

Rajkhowa, C.; Gogoi, A.R.; Borkakoty, M.R. and Das, M.R. 1992a. Incidence of schistosomes in cattle, buffaloes and goats in Assam. *Indian Vet. J.* **69**: 273-275.

Rajkhowa, C.; Gogoi, A.R.; Borkakoty, M.R. and Sharma, B.C.1992b.Some observations on haematological changes in experimental *Schistosoma indicum* infection in cattle. *Indian J. Anim. Sci.* 62(2):125-126.

Rao, M.A.N. 1933. A preliminary report of the successful infection with nasal schistosomiasis in experimental calves. *Indian J. Vet. Sci. Anim. Husb.* **3**: 161-162.

Rao, M.V.G. 1947. Schistosomiasis among sheep/goats in Poona R.I.A.S.C. butchery. *Indian Vet. J.* **24**: 11-13.

Satyanarayanacharyulu, N.; Rao, T.S. and Christopher, K.J. 1969. *Schistosoma indicum* in an elephant. *Curr. Sci.* **38**: 344.

Sayal, N.N. 1964. Studies on ovine helminthic hepatopathy. M.V.Sc. Thesis, JNKVV Jabalpur. (Cited Jain, P.C. 1972).

Seubert J.; Pohlke, R. and Loebich, F. 1977. Experientia. 33: 1036-1037 (Cited Groll, E. 1984).

Sharma, D.N. and Dwivedi, J.N. 1976. Pulmonary schistosomiasis in sheep and goats due to *Schistosoma indicum* in India. *J. Comp. Path. Ther.* **86**: 449-454.

Sharma, G.D. and Dadhich, Hemant. 1998. Pulmonary schistosomiasis in camel - a case report. *Indian J. Vet. Path.* **22**(1): 71-72.

Singh, Jagjit; Gupta, R.P. and Kaushik, R.K. 1985. Report of an outbreak of *Schistosoma indicum* in sheep in Haryana. *Indian Vet. Med. J.* **9**: 223-224.

Singh, K.P. and Parihar, N.S. 1988. Pathology of fluke infestation in livers of sheep and goats. *Indian J. Anim. Sci.* **58**(8): 890-894.

Singh, S.V.; Mann, H.S.; Sharma, D.N.; Srivastava, A.K. and Prakash, D. 1995. Pulmonary schistosomiasis in sheep and goats : incidence and pathomorphology. *Indian J. Vet. Path.* **19**(1): 38-39.

Sircar, R.N. 1956. Helminthic diseases of sheep in Bihar and their control. *Indian Vet. J.* **33**: 111-119.

Skrjabin, K.I. 1913. *Schistosoma turkestanicum* nov. sp. cin neuer Parasit des Rindes aus Russiseh-Turkestan. Zeitschrift fur Infection Skrankheiten, Parasitare, Kramkheiten und Hygiene der Haustiere, **13**: 457-468 (Cited Kumar, V. and de Burbure, G.1986).

Soulsby, E.J.L. 1982. Techniques. In Helminths, Arthropods and Protozoa of Domesticated Animals. 7th edn. ELBS and Bailliere Tindall, London, 763-778.

Srivastava, H.D. and Dutt, S.C. 1951. Life history of *Schistosoma indicum* (Montgomery, 1906). A common blood-fluke of Indian ungulates. *Proc. 42nd Indian Sci.Cong.* **III:** 353

Srivastava, H.D. and Dutt, S.C. 1962. Studies on *Schistosoma indicum. I.C.A.R. Research Series No.* 34, 91 pp., New Delhi.

Srivastava, H.D.; Murlidharam, S.R.G. and Dutt, S.C. 1964. Pathogenicity of experimental infection of *Schistosoma indicum* Montgomery (1906) to young sheep. *Indian J. Vet. Sci. Anim. Husb.* **34**: 35-40.

Srivastava, M.K. and Agrawal, M.C. 1999. Doubtful efficacy of lithium antimony thiomalate (Anthiomaline) in schistosomosis. *J. Vet.Parasitol.* **13**(1): 67-68.

Steel, R.G.D and Torrie, J.H. 1960." Principles and Procedures of Statistics." Mc. Graw Hill Book Co., Inc. 1960.

Steiner, K.; Garbe, A.; Dickmann, H.W. and Nowak, H. 1976. *Eur.J. Drug Metab. Pharmacokin.* **1**: 85-95.

Supekar, P.G. and Jain, P.C.1977. *Schistosoma indicum* infection in a Gir bullock, a case record. *Livestk. Advis.* **2**: 41-42.

Upatoom, N.; Horchner, F. and Leidl, K. 1988. Therapy against *Schistosoma spindale* infection in cattle and buffalo. *Vet. Med. Rev.* **59**: 171-174.

Vashishta, M.S.; Jasbir Singh; Pant, U.V.; Kulshrestha, T.S. and Nathu Singh. 1981. Discovery of a new focus of *Schistosoma indicum* infection: Some observations on emergence of natural infection in sheep with inhabitance of parasite also in lung, that respond to lithium antimony thiomalate. *Livestk. Advis.* **6**(1): 57-59.

Verma, A.K. 1954. Studies on the nature, incidence, distribution and control of schistosomiasis in Bihar. *Indian J. Vet. Sci. Anim. Husb.* **24**: 11-31.

Vyas, I.; Sharma, G.D. and Vyas, U.K. 1992. Hepatic vascular lesions in caprine schistosomiasis. *Indian J. Anim. Sci.* **62**(1): 45-47.