PATHOGENESIS OF EXPERIMENTAL SCHISTOSOMA BOVIS IN GOATS AT DIFFERENT LEVELS OF INFECTION

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A Thesis submitted in fulfillment of Degree of Master of Science (Zoology)

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August 2004
DEDICATION

To my family

And to all whom I love,

Respect and appreciate
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ACKNOWLEDGEMENT

The work described in this thesis was carried out in the parasitology Department of Central Veterinary Research Laboratories (CVRL), Soba during the period March 2001 to May 2003. I am indebted to the Director of (CVRL) my supervisor, Professor Ali M. A/Majid for financing my studies and giving me the opportunity of working in his department, closely following this work with guidance, unfailing help, diligence, keen interest and patience.

I am very grateful to my co-supervisor, Dr. Osman Fadul for his continuous guidance and help in the preparation of this thesis.

I would like to express my deep gratitude and thanks to Dr. Ibtisam Amin Goraish, Head Department of Parasitology (CVRL) for provision of facilities and encouragement.

Deep appreciation is extended to Dr. Zakia Abbas, Head, Department of Pathology (CVRL) for her valuable assistance.

I am grateful to the staff of University of Khartoum, Faculty of Science, Department of zoology for their valuable cooperation.

My sincere thanks are also due to the following:

The staff of Parasitology Department (CVRL) for collecting snails from the field and help in maintaining them in
the laboratory and for their technical help, and continuous assistance.

The staff of the Department of Pathology (CVRL) for their technical assistance and photography.

The staff of the Department of Biochemistry (CVRL) for technical assistance.

Mrs. Samira Amin for Typing the thesis.
Special thanks

To

Central Veterinary Research Laboratories
(CVRL) Soba

Department of Parasitology

The Main Sponsor for This Work
ABSTRACT

*Schistosoma bovis* is an important veterinary and economical problem in the Sudan and other African, Mediterranean and Middle Eastern countries. Schistosomiasis causes severe outbreaks associated with high mortality rates among cattle, sheep and goats.

Experimental work on schistosomiasis involved the establishment of the complete life cycle of the parasite under laboratory conditions. An active breeding colony of the suitable snail host was established to provide a steady supply of cercariae for goats’ infection. The effect of experimental *Schistosoma bovis* infection on the clinical and pathological alternations was investigated in male goats (10-12 month old). Twenty goats were divided into four groups A, B, C and D. Body weight and haemogram were measured for every week of experiment. Each animal in group A, B and C was infected with 500, 2000 and 5000 *Schistosoma bovis* cercariae percutaneously respectively. Animals in group D were kept as uninfected control.

Serum and faecal samples were collected after infection. The experimental goats were slaughtered by the end of the experiment (27 weeks after infection) for worm recovery and tissue egg count. The representative tissue portions were fixed and processed routinely for histopathology. Infected goats developed clinical signs of illness 6 to 8 weeks for group A, B and C
respectively. These included inappetance, dull appearance, general weakness and sunken eyes. The appearance of symptoms coincided with the start of oviposition and passage of schistosome eggs in faeces. The results obtained showed significant decrease between the infected groups in body weights, haemoglobin concentration, total RBC counts and albumin values whereas total WBC counts, total protein and globulin showed no significant increase. Faecal egg counts, worm recovery and tissue egg counts showed significant changes associated with the level of infection.

The main histopathological findings in the livers of infected animals were granuloma formation and hepatocellular swelling and vacuolation. Ova granuloma were also noticed in the lung, lymph node and intestine. Glomerulo-inertial nephritis was observed in the kidneys. Haemosiderin pigment was deposited in the spleen. In the heart, myocarditis was observed. These lesions were frequently encountered in tissue of animals of group C followed by group B and were scarcely detected in tissues of group A.
CHAPTER ONE

Introduction and General Review of Literature

Schistosomiasis is a common parasitic infection of man and domestic animals in many tropical and subtropical parts of the world. In domestic animals, the disease is of widespread nature and severe epizootic with heavy mortalities had occasionally been reported in the Sudan (Eisa, 1966, Hussein, Majid et al., 1980a). while most other communicable diseases are coming under control, schistosomiasis is increasing and had recently been introduced into areas known to be schistosome-free (Majid, personal communication). This increase is primarily due to the extensive development of water resources for agricultural expansion. These man-made habitats provided suitable breeding conditions for the snail intermediate hosts with the consequent increase in the prevalence and intensity of infection.

In the Sudan, no species of domestic animal schistosomiasis other than Schistosoma bovis is known to exist, although Malek (1969) suggested that S. mattheei probably occurs in the southern region since the suitable snail vectors were present and because of the prevalence of this parasite species in the neighboring countries.

S. bovis was first reported in the Sudan in 1915 when an infected cow was recorded in the White Nile Province (Anon, 1915). Several epizootics of S. bovis infection were described among cattle and sheep causing heavy economical losses (Eisa,
1966, Hussein, 1968) and the parasite was also shown to occur in the western and eastern provinces and also in the southern region (Malek, 1969, Elbadawi and Slepnov, 1976). Epizootics have recently spread to areas, which were known to be free of the disease like Khartoum State (Majid, unpublished observations).

The morphology of *S. bovis* conforms to the general features of its family and was first extensively studied by Khalil (1924) who made detailed description of the parasite using specimens from Sudan and Egypt. His description was later modified by MaChattie and Chadwick (1932) who examined around 4000 specimens from Iraq.

The mature egg of *S. bovis* is non-operculate, fusiform or spindle-shaped and broad in the middle. It tapers to both ends with one end bearing a spine which may be 15µ long (Lengy, 1962a) and the other end is rounded off at its extremity. It measures 231-258µ by 66-70µ. The uterine eggs are found in rows or arranged in tandem fashion and range from 13-57 eggs in the uterus (Dinnik and Dinnik, 1965).

1.1. **Transmission of *S. bovis***:

The transmission and life-cycle of *S. bovis* are similar to those of other species of mammalian definitive host. All the snail vectors of schistosomes are aquatic ones living in fresh water except for the amphibian snails of the genus *Oncomelania* which transmits *S. japonicum*. 
*S. bovis* is transmitted by snails of the genus *Bulinus*. In the Sudan it is mainly transmitted by *B. truncatus* (Malek, 1961) and other species such as *B. africanus*, *B. ugandae* and *B. forskaliū* have also been reported to carry natural or experimental infections (Majid et al., 1980a). The infectivity of *S. bovis* in these snail hosts probably differs with the different geographical strains of the parasite. The strains of *S. bovis* from Morocco, Sardinia, Iran and Kenya had different infectivities to five species complexes of *Bulinus* snails. At least two distinct biological forms of *S. bovis* existed: the truncatus-borne *S. bovis* occurring in north Africa, some Mediterranean areas and the Middle East which was highly compatible with the truncatus species group but of low compatibility with the forskalliū and africanus group and the forskaliū and africanus-borne *S. bovis* from central and east Africa which was highly compatible with the truncatus, africanus and forskaliū species groups.

1.2. Life-cycle:

The life cycle of schistosomes was first extensively studied in the case of the human parasites. That of *S. bovis*, on the other hand, was first investigated briefly by Brumpt (1930) and later investigated in more detail by Lengy (1962b).

The mature eggs that are passed in the faeces of infected animals hatch in water releasing the miracidium. This process of hatching is brought about by osmotic effects and active miracidial movements which result in bursting of the shell at the centre of
the egg releasing the miracidium. Other factors such as light, temperature and salinity are essential for hatching. The miracidia of *S. bovis* are positively phototropic and negatively geotropic and it was found that in the absence of light hatching is suppress but not completely inhibited (Lengy, 1962b). When newly hatched miracidia encounter a suitable snail host, they burrow through its soft tissue assisted by active movement of the miracidium and also by histolytic enzyme and there seems to be a chemotactic attraction from the mucous secretion of the snails (Wright, 1959) which enables the miracidium to locate nearby hosts. Only when a suitable snail host is invaded, however, does further development take place; otherwise, the miracidium is subjected to tissue reactions from the snails, become walled off by these reactions and subsequently been destroyed by amoeocytes (Wright, 1967).

Within the snail host, certain metamorphic changes take place as a result of which the miracidium first changes into mother sporocyst. This stage divides further giving several daughter sporocysts which in turn multiply to produce cercariae. The latter begin to leave the snail’s body as early as the third week post-infection. Cercarial eruption is also enhanced by the presence of light. Lengy (1962b) found that in the case of Israeli *S. bovis*, 1500-2000 cercariae may be produced from a single snail (*B. truncatus*) within a 24 hours period. When a suitable mammalian host is encountered, the cercaria applies its cephalic end to the skin of the host and by active mechanical movement as
well as histolytic enzymes, secreted by its penetration gland, it thrusts into the skin leaving its tail behind. Once get into the skin it transforms to a schistosomulum or post-penetration larva. *S. bovis* schistosomula usually remain in the skin and subcutaneous tissue of the mammalian host for about 24-48 hours, then penetrate the blood or lymph vessels and are carried to the heart and then to the lungs capillaries.

In the lungs, further development of the schistosomula takes place before they migrate to the liver. The route of migration from the lungs to the liver is, however, controversial and two routes were suggested by the different investigators. One route is that these larvae leave the pulmonary capillaries, migrate through the lung tissues and pass through the diaphragm, entering the liver through its surface. The alternate route is that the schistosomula are transported via the pulmonary vein and subsequently via the arterial circulation to the hepatic portal system (Hussein *et al*., 1975).

Lengy (1962a) suggested that, unlike, *S. japonicum*, there is no cuticular spirlation in the cercariae of *S. bovis* and that the latter have blunt anterior ends and show no trace of penetration glands. Therefore, after penetrating the skin “it is more feasible to assume that the larvae are transported to the liver with the blood circulation”. Detailed and serial histopathological studies on mice infected with *S. bovis* and *S. mattheei*, however, suggested that
these schistosomula migrate directly through the pulmonary alveolar parenchyma, into the pleura, piercing the diaphragm and entering the liver through its surface (Hussein, 1968, 1972a). On the other hand, some authors (Georgi et al., 1983) using autoradiography with radioselenium–labelled cercariae, suggested that both routes of migration from the lungs to the liver were possible.

In the liver the parasites grow further and more rapidly to attain sexual maturity, when they mate and move to the mesenteric veins. The cercariae arising from one miracidium will mature into a single sex being either a male or female worm and this is genetically inherited. Schistosomes do not multiply in the definitive host and for this reason the intensity of infection depends on the number of cercariae invading the host.

1.3. Prepatent period:

The prepatency of *S. bovis* seems to vary with the strain of the parasite and the species of the mammalian host. Lengy (1962a) reported a prepatent period of 35-42 days in mice. In the Sudan, Malek (1961) reported that it was 75 days in cattle, while in *S. bovis* from the white Nile the prepatency in cattle was found to be 42-60 days (Majid, 1969). Hussein *et al.* (1975) found prepatency of Sudanese *S. bovis* in calves and sheep to be 7 weeks.

After maturation the gravid female moves to deposit its eggs in the small intestinal venules and the eggs extrude the blood
vessels, perforate the intestinal wall leaving to the outside environment with the faeces of the infected host. A large proportion of eggs is, however, retained in the wall of the alimentary tract or drained through the portal circulation to become entrapped in the small intrahepatic portal radicles, thus accounting for the pathological lesions encountered in the liver. In ruminants, however, the excretion of eggs through the intestinal wall is much more important from the clinicopathological standpoint since it produces haemorrhages and the consequent blood and plasma protein losses.

1.4. Pathogenesis, clinical manifestations and pathology of *S. bovis*:

During prepatency, few clinical manifestation are seen, and there is only a slight drop in the packed cell volume and haemoglobin levels which arise from the haemodilution resulting from the slight expansion of the plasma volume of infected animals (Bushara *et al.*, 1980). The acute form of the disease, which coincides with the onset of oviposition, on the other hand, is characterized by a mucoid and bloody diarrhoea due to intestinal haemorrhage induced by egg extrusion though the intestinal wall.

At the same time, the animal shows signs of anorexia, listlessness, dehydration and progressive loss of weight coupled with marked eosinophilia. Hussein (1968) reported a biphasix rise in eosinophil counts in calves experimentally infected with *S.*
bovis: the first eosinophil peak being associated with the onset of oviposition and the second peak arising later when some of the worms die in the animal’s body. Others reported eosinophilia in experimentally infected sheep, goats and calves (Saad, 1979). This acute stage is also manifested by a normocytic normochromic anaemia, hypalbuminaemia and hyperglobulinaemia, which were reported in cattle (Hussein and Tartour, 1973, Hussein and Amin, 1973, Majid et al., 1980b) and in sheep (Gameel, 1974, Saad, 1979, Majid et al., 1983).

Similar manifestations were also reported in sheep infected with S. mattheei (Preston and Dargie, 1974) and also in cattle infected with this parasite (Hussein, 1968, Lawrence, 1980). Besides, there is a marked decrease in PCV and red cell counts. Saad (1979) using radio-isotopic tracer techniques showed that in cattle and sheep experimentally infected with S. bovis there was an accelerated rate of red cell breakdown due to its loss from the circulation. This was first clear at the beginning of patent infection at seven weeks post exposure, and resulted from intestinal haemorrhage, then became progressively more severe during the subsequent two months, before subsiding. There was also hypoalbuminaemia due to increased rate to albumin catabolism with marked depletion of albumin levels particularly from the extravascular pool. Saad (1979) also showed that erythropoiesis was increased as a result of red cell breakdown but this was inadequate to compensate for blood loss and there was
also an increase in the plasma volume and the total body water content.

The pathology of natural and experimental *S. bovis* in cattle, sheep and goats was described by a number of investigators (Mustafa and Hussein, 1967, Gameel, 1974, Saad, 1979 and Majid *et al.*, 1980b). In the liver, greyish or whitish necrotic foci or granulomas containing eggs or their ruminants are seen. These lesions occur in the subcapsular region and on the cut surface of the liver and consists histologically of epithelioid cells, lymphocytes, eosinophils and multinucleated giant cells. The lesions may also show areas of necrosis and/or ‘Hoepli’s reaction. Periportal inflammatory infiltration, fibrosis and the deposition of a dark granular schistosomal pigment in the portal tracts and Kuppfer cells are also constantly observed. There is progressive and marked thickening of the portal tracts with extensive vascular lesions as the disease advances. Such vascular lesions include extensive medial lymphoid nodules and follicles containing dead eggs and worms in the portal veins and were first reported by Sobrero (1958) in Somali cattle.

The alimentary tract shows variable pathological manifestations depending on the duration and severity of infection. Several authors described variable catarrhal inflammatory changes (Hussein, 1971; Gameel, 1974; Hussein *et al.*, 1975; Majid *et al.*, 1980b). Similar findings were reported in *S. mattheei* infections (McCully and Kruger, 1969, Van Wyk *et al.*, 1980b).
The excessive damage caused by egg excretion through the intestinal wall is manifested by haemorrhages, catarrhal inflammation and epithelial laceration with the consequent infiltration of inflammatory cells in the mucosa and submucosa, formation of granulomas and progressive fibrosis. Vascular lesions such as intimal proliferation, medial hypertrophy and perivascular infiltration of subserosal and submucosal veins are also commonly encountered.

As the disease advances these lesions become progressively fewer and focal in nature indicating partial resolution. Saad (1979) found that in two groups of calves infected with 100 and 200 cercariae per Kg body weight, the hepatic and intestinal lesions were more marked in the latter level of infection. He also found that granulomatous and eosinophilic reactions were more pronounced in the small intestine but as the disease progressed they were also observed with increasing frequency in the large intestine. This suggested partial migration of the parasites from the small to the large intestine.

Other organs involved include the pancreas where adult worms might be found in the blood vessels causing various changes such as proliferation in the Tunica intima, medial hypertrophy, cellular reactions in addition to formation of granulomas around eggs in the interstitium and parenchyma of this organ. Pulmonary lesions may also be found such as adult worms in the pulmonary blood vessels as well as eggs with or
without surrounding tissue reaction or granulomas in the lung parenchyma and a variety of pulmonary vascular lesions. The lung was may also show greyish areas of discolouration with various stage of pneumonia and pigmentation. The portal and mesenteric lymph nodes may be swollen and caseated and in chronic stage also show areas of fibrosis and calcification as they are also common sites of oviposition.

The deleterious effect of the parasite on the bodyweight has been attributed partly to inappetance and partly to pain and the increased peristaltic movement leading to diarrhoea (Lawrence, 1977). Berry et al. (1973) working on S. mattheei also ascribed the weight loss in ruminant schistosomiasis to muscle wasting because of the mobilization of the muscle proteins to compensate for the protein loss through haemorrhage into the intestinal lumen. Furthermore, the inflammatory changes in the intestinal tract result in impaired digestion and malabsorption. The irritation of nerve endings and the increased intestinal activity may all contribute to the body weight loss (Saad et al., 1980).

With the progress of the disease and as the animal passes the acute phase, many of the clinical and pathological manifestations may resolve. Egg output and worm burden thus decrease and, consequently, the associated clinical and pathological sequence improve.

Now the disease runs a more chronic course and although the earlier set-backs arising from the acute stage are never fully
compensated, further manifestations of infection become less marked. Majid et al. (1980a) reported that in the White Nile area the brunt of clinical disease is associated with young calves 6-18 months old with a subsequent drop in the intensity of infection with advancing age. In this respect, cattle schistosomiasis is a well-adapted parasitic infection where once the acute phase is passed, the parasite and the host live in a state of natural balance.

1.5. Diagnosis of *S. bovis*:

Ruminant schistosomiasis resembles many other debilitating and long-standing infections especially in its chronic form. History and clinical signs alone are, therefore, inadequate and diagnosis should be confirmed by demonstrating *S. bovis* eggs in the faeces of the suspected animal.

Parasitological techniques such as sedimentation and hatching techniques have been described and can be quite useful. They are limited, however, by the fact that in chronic infections the very few eggs passed in faeces may fail to be detected and that the hatched miracidia cannot distinguish the species especially in areas where more than one schistosome is present. A quantitative technique was described by Pitchford and Visser (1975) and has been used in the present study. Although this technique is moderately sensitive, it cannot detect very light infections and is not used in routine diagnosis in this country. Other techniques such as rectal scraping and liver biopsy have been employed in man with less usefulness in diagnosing animal schistosomiasis.
Immunodiagnosis by various serological tests was attempted in human schistosomiasis with variable degree of success. Some inconclusive results have been obtained with the complement fixation and fluorescent antibody technique (Hussein, 1972b). Most of the routine serological testes currently available are not highly specific to distinguish between recovered and actively infected individuals. Over recent years (Dalton et al., 1996) succeeded in isolating a schistosome-specific antigen for *S. mansoni* which may pave the way for the development of a more sensitive technique for the immunodiagnosis of schistosomiasis.

1.6. The objectives of the present study:

Infection with *S. bovis* is quite prevalent among livestock in the Sudan. Due to agricultural expansion and some changes related to climatic factors and changes in the animal movement, the well known situation of *S. bovis* has dramatically changed. It was thus reported that occasional epizootics of *S. bovis* in cattle had occurred in Khartoum State in the mid-nineties, especially in foreign and cross-bred animals. Khartoum has been considered until recently free of schistosomiasis. It is therefore, imperative to obtain information on how this area-specific trematode affect animals. This is essentially required for small ruminants. Very little work had been carried out in sheep (Majid et al., 1983) and even less so in goats.

As it has been stated earlier that schistosomes do not multiply in the infected host and for this reason the pathogenesis
of the disease is very much related to the infective dose. In cattle, Saad (1979) had examined the pathophysiological consequences of two levels of infection (100 and 200 cercariae per kg body weight). The objective of this study is to investigate the clinical and pathological alterations imposed by *S. bovis* on goats. Parameters such as clinical symptoms, bodyweight and haemogram as well as pathology of the infection in the different organs are to be examined. These changes are related to different cercarial levels of infection. The execution of the study is, therefore, designed to provide an insight into the pathogenesis of *S. bovis* under low, medium and massive doses of infection in goats.
CHAPTER TWO
Materials and Methods

2.1. Snails collection:

Field snails were collected by scoop from slow running water at irrigation schemes in Dowar Elmahade and Elgened area. They were transported to the laboratory in plastic barrels containing water from the snail’s habitat. In the laboratory, the snails were washed with dechlorinated tap water and transferred to plastic dishes. The snails were screened individually for natural infection and the infected snails were discarded while the non-infected snails were kept in plastic dishes. The dishes were cleaned from feed, faecal materials and other debris twice a week by using a sieve and the water was changed once a week. The snails were fed on dried lettuce.

2.1.1. Preparation of Miracidia:

From naturally infected calf with *S. bovis*, the faeces were collected and processed by sedimentation using normal saline then distilled in a conical flask which was put under artificial light for about half an hour to induce hatching. The miracidia accumulated at the top of the flask.

2.1.2. Infection of snails with Miracidia:

Snails were infected by placing them in small glass tubes containing dechlorinated tap water to which 10-15 miracidia were transferred by automatic pipette. The snails were exposed to
infection over night, then maintained in plastic dishes and kept in dark. The snails were examined for the presence of cercariae after 3-4 weeks post infection.

2.1.3. Shedding, collection and counting of cercariae:

Positive snails were removed early in the morning, placed in beakers, washed repeatedly from faecal and feed debris and suspended in a sufficient volume of water. The snails were exposed to artificial light for 30-60 minutes to stimulate cercarial shedding. For counting the cercariae, three 0.1ml samples were taken by automatic pipette (Gibson) placed on a glass petri dish, fixed and stained with lugol’s Iodine. The cercariae in each sample drop were counted using a dissecting microscope. The number of cercariae per ml was counted according to the following formula:

\[
\text{No. of cercaria in all samples \times 10} \\
\frac{3}{3}
\]

2.2. Experimental animals and design:

Twenty male goats aged about 10-12 month were purchased. The animals were examined for internal parasites and treated period of seven weeks for the presence of schistosome eggs in their faeces and were found to be negative. The goats found to be infected with coccidia and intestinal nematodes, were drenched with (sulphonamide 1.5mg/kg) and Albendazole (400mg/kg). As a further precaution, all animals were drenched with the same drug 4 weeks before the commencement of the investigations. The goats were divided into 4 groups (A, B, C and
D) of approximately equal body weight. Group A consisted of 5 animals exposed to 500 *S. bovis* cercariae per kg body weight, group B consisted of 5 animals exposed to 2000 *S. bovis* cercariae per kg body weight and group C consisted of 5 animals exposed to 5000 *S. bovis* cercariae per kg body weight. The remainder group D acted as uninfected control. The goats were maintained indoors within the premises of the Central Veterinary Research Laboratory at Soba, allowed free access to water and fed on straw supplemented with a concentrate. The goats were ear-tagged, kept in clean pens. The goats in each group were weighted regularly till the end of the experiment, faecal and blood samples were collected regularly and examined.

**2.3. Collection and analyses of blood samples:**

The blood samples were collected weekly by jugular vein puncture, 5ml of blood were obtained from each animal. The blood sample was divided into two portions, about 2ml was placed in a clean dry tube, containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant for the haematological analyses. The use of EDTA as anticoagulant was preferred being suitable because it kept the size of erythrocyte constant, so that the determination of haematological indices (MCV, MCH and MCHC) were more accurate. The determinations of haematological values have been done by the methods described by Baker and Silverton (1980).
The second portion of the blood sample (3ml) was left undisturbed for 7 hours at room temperature in order to clot for serum collection. Haemolysis-free serum was pipette into a clean vial and kept frozen at −20°C for subsequent analysis.

2.4. Haematological methods:

2.4.1. Haemoglobin concentration (Hb):

Hemoglobin concentration was determined by cyanomet-haemoglobin technique using a haemoglobimeter (Corning Ltd., UK). The haemoglobin concentration was measured in g per 100 ml of blood and then converted to g per L.

2.4.2. Packed cell volume (PCV):

The PCV, expressed as percentage of whole blood, was determined by the microhaematocrit method using a special centrifuge (Gawksley, England):

The capillary tube (7.5mm x 1.0mm) was filled by capillarity from well-mixed blood sample up to ¾ the length of the tube. The outside of the tube was carefully cleaned with absorbent gauze and the vacant end was sealed with special clay (Cristaseal). The filled tube was placed in the slot on the head of high speed centrifuged with the open end towards the bulb and sealed end as close as possible to the rim of the head. The centrifuge was covered and spun at 3000r.p.m. for 5minutes. Hawksley microhaematocrit reader was used to obtain PCV (%).
2.4.3. Total White Blood Cell Counts TWBCs):

The blood sample was thoroughly mixed then 0.02 ml was pipetted and discharged into a test tube containing 1ml of the leukocyte diluting fluid (Turk’s fluid” composed of glacial acetic acid 1ml, 1% aqueous gentian violet 1ml, distilled water up to 100ml). The pipette was rinsed thoroughly with the diluting fluid to ensure removal of all traces of blood. The test tube was covered with a rubber bung and mixed thoroughly by slow inversion so that a dilution of 1:20 was obtained.

The improved Neubauer haemocytometer and the coverslip was pressed on the surface of the haemocytometer. The diluted blood counting chamber and the cells were allowed to settle.

Under low power, using x10 objective of the bright field microscope. The number of white blood cells was counted in each of the 4 large corner squares. The rule for including and excluding cells touching the lines was same as for erythrocyte count.

The calculation was made by multiplying then umber of cells (N) counted by both dilution factor and volume factor.

Each large corner square has an average area of 1mm$^2$ and a depth of 0.1mm, giving a volume of 0.1µl. Since 4 large squares were used for counting the total volume was 0.4µl because the standard volume used in cell counts is 1ml, this should be multiplied by 2.5. The dilution factor is 20, then as the 4 squares have been counted, the total count was obtained as follows:
Total leukocytes count ($x10^3/\mu l$)=
\[\frac{N \times 1 \times 20}{0.4} = N \times 2.5 \times 20 = N \times 50\]

2.4.4. **Total Red Blood Cell Counts RBCs:**

The blood sample with anticoagulant was carefully mixed. Blood was drawn up to 0.02 mark of the Thoma Red Cell pipette by gentle suction. The tip of the pipette was wiped out using clean cloth. The sample was discharged into the test tube containing 4ml of erythrocyte diluting fluid (Gower’s fluid: sodium sulphate ‘anhydrous’ 12.5gm glacial acetic acid 33.3ml, distilled water 200ml). The pipette was thoroughly rinsed with diluting fluid to ensure removal of all traces of blood. The test tube was covered with a rubber bung and the contents were mixed thoroughly by slow inversion. The dilution of blood obtained was 1:200.

Improved Neubuer haemocytometer was used for counting erythrocyte. The haemocytometer was used for counting erythrocyte. The haemocytometer and coverslip were cleaned; then breathing on the haemocytometer, the coverslip was pressed on tightly using the thumb. The diluted blood was mixed and a capillary tube was used to fill the space between the counting chamber and the coverslip, taking care that no fluid flows into surrounding moats. About 5 minutes were allowed for the cells to settle.
Using high power (x45) objective of the bright field microscope, all the red cells were counted in 5 squares in the central area. Each of the 5 small squares is divided into 16 smaller squares; a total of 80 of these small squares were counted.

The calculation of the erythrocyte per microlitre (µl) was made by multiplying the number of cell counted (N) in 80 small squares by both the dilution factor and the volume factor. Each small square has an area of $\frac{1}{400} \text{ mm}^2$ and a depth of $\frac{1}{10}$, giving a volume of $\frac{1}{400} \mu l = \frac{1}{50} \mu l$.

Because the standard volume used in cell counts in 1 µl, this volume should be multiplied by 50. The dilution factor was 200 and accordingly the erythrocyte count was obtained as follows:

The erythrocyte count (millions/µl) =

\[= N \times 50 \times 200\]
\[= N \times 10,000.\]

**2.4.5. The haematological indices (mean corpuscular values):**

Utilizing the values obtained for total erythrocyte count, haemoglobin concentration and packed cell volume (PCV), it was possible to calculate the volume of an average erythrocyte and its haemoglobin concentration.

**2.4.5.1. Mean corpuscular volume (MCV):**

This was determined from the PCV and the red cell count:

\[MCV = \frac{PCV \times 10 \times (F1)}{RBC \times 10^6/\mu l}\]
2.4.5.2. Mean corpuscular haemoglobin (MCH):

This index expresses pictograms the weight of the haemoglobin in the average erythrocyte:

\[ \text{MCH} = \frac{\text{Hb (g/dl)} \times 10 \, (\text{Pg})}{\text{RBC} \times 10^6 / \mu l} \]

2.4.5.3. Mean corpuscular haemoglobin concentration (MCHC):

This index measures the ratio of weight of haemoglobin to the volume of erythrocytes and the expression is in grams per deciliter:

\[ \text{MCHC} = \frac{\text{Hb (g/dl)} \times 1000 \, (\text{g/dl})}{\text{PCV}} \]

2.5. Biochemical techniques:

2.5.1. Determination of serum total protein:

The concentration of total serum proteins was determined using Biruet reagent as described by Weichselbaum (1946):

**Principle:**

Copper in alkaline solution reacts with peptide bonds of amino acids in protein producing a violet colour.

Biuret: \( \text{NH}_2\text{-Co-NH-Co-NH}_2 \)

One copper atom complexes with four molecules of Biuret, the linkage being to the central nitrogen atom, the shade of column being different with different proteins.
**Reagents**

**Biuret reagent (stock reagent):**

Nine grams of sodium potassium tartrate were dissolved in 500ml of 0.2N NaOH. Three grams of copper sulphate (CuSO₄, 7H₂O) were dissolved. Then 5g of potassium iodide were added and the volume was made up to 1 litre with 0.2 N NaOH.

**Colour reagent:**

From stock reagent, colour reagent was prepared by diluting 50ml of stock reagent to 250 ml with 0.2 N NaOH solution.

**Procedure**

<table>
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<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
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<tbody>
<tr>
<td>Distilled water</td>
<td>3ml</td>
<td>-</td>
<td>2.8ml</td>
</tr>
<tr>
<td>Standards</td>
<td>-</td>
<td>3ml</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>0.2ml</td>
</tr>
<tr>
<td>Biuret reagent</td>
<td>5ml</td>
<td>5ml</td>
<td>5ml</td>
</tr>
</tbody>
</table>

Mixed and incubated for 10 minutes in water bath at 37°C read at 540nm using spectrophotometer.

\[ T \times 7.5 = g/100ml \]

\[ S \]

Where: \( T = \) Sample \quad \( S = \) Standard.

**2.5.2. Determination of serum albumin:**

Serum albumin level was determined by the colorimetric method.
**Principle:**

This method depends on dye binding Bromocresol green (BCG) is the best binding reagent that gives green colour with albumin at low pH 4.2.

**Reagents:**

0.174g of bromocresol green was dissolved in 2.5 ml of 0.1 N NaoH and the volume was made up to 25ml with distilled water. 6ml of bromocresol green added to 17.3 ml of 29.4% molar sodium citrate and 32.7ml of 21% molar citric acid. The volume was made up to 1 litre with distilled water and the pH was adjusted to 3.8.

**Procedure:**

<table>
<thead>
<tr>
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<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
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<tbody>
<tr>
<td>Standards</td>
<td>-</td>
<td>0.2ml</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>0.02ml</td>
</tr>
<tr>
<td>BCG</td>
<td>4ml</td>
<td>3.8ml</td>
<td>4ml</td>
</tr>
</tbody>
</table>

Mixed incubated at room temperature for 5 minute. Read at 630nm:

\[ T \times 5 \text{ concentration} = \frac{g}{100ml} \]

Where \( T = \) Sample \hspace{1cm} \( S = \) standard.

**2.5.3. Determination of globulin:**

Was done by subtracting the values of albumin from total protein.
2.6. Parasitological techniques:

2.6.1. Infection of goats:

The skin was prepared by shaving an area of about 15 cm diameter of abdominal skin just posterior to the last floating rib, without anesthesia (Hussein, 1971). The shaved part was then thoroughly washed with tap water. Before applying the cercarial suspension. The animals were casted and immobilized by two assistants while a cylindrical metal container 7-10cm diameter opened at both ends, was applied firmly to shaved skin. Cercariae suspended in about 100-200 ml of water were added to the metal container. Successfully penetration was later judged by the number of tails found microscopically in the recovered cercarial fluid.

2.6.2. Faecal egg counts:

These were based on the method of Pitchford and Visser (1975) with the following modifications. Samples of about 5 grams of fresh faeces were obtained from the rectum and placed in small uniform thin walled plastic containers with well-filled lids. The faeces and containers were weighed, almost fitted with 10% formalin, and left to stand for at least 48 hours. The whole of the weighed sample plus formalin were poured over a wire fresh 240µm flitted on a large funnel leading to inner nylon filter 95µm of pitch-ford’s apparatus. A jet of water from a hose adjusted with the fingers to give a spray or jet was applied to break up the faeces. Eggs and small faecal debris passed through
the walls of the inner filter into outer one while the other debris was returned in the inner filter. Any blockage of the filter walls was rectified by spraying onto the walls of the outer wall of the inner filter with the apparatus in situ (Plate 1).

The contents of the outer filter were collected in numbered plastic cans by opening the tap at the bottom of the outer filter and spraying its walls with water. Faeces in the inner filter were washed 4-5 times with removed each time to the outer filter contents into the can. Each plastic can was filled with tap water to a known volume (1050ml) and its contents stirred by inverting the can several times. Three 15 ml samples were taken quickly using a measuring cylinder. Each sample was placed in a numbered 80 ml thick-walled plastic container. 2.5ml of stock acid fuschin dye (1% acid fuscin solution; 2.8gm Na(OH) in 2220 ml distilled water, 4ml conc. Hcl in 4550ml distilled water) was added to the contents of the containers and each heated in a water bath at 70-75°C for 5 minutes. The containers were removed and the samples decolourized by added stock NaOH solution until the colour disappeared. Stock Hcl solution was then added until the colour re-appeared, and the contents poured through Whatmann No. 4 filter paper in a porcelain filter funnel attached to the vacuum pump (Plate 2).
Plate 1: Showing the Pitchford apparatus for counting *S.bovis* eggs in faeces:

a) Funnel holding wire mesh.
b) Pitchord’s filtration apparatus.
c) Hose connected to water pipe.
**Plate 2:** Showing vacuum pressure pump for processing the faecal filtrate:

a) Vacuum pressure pump container.

b) Vacuum pump.
The contents were then pumped down and further stock NaOH followed by stock HCl was added to the filter paper and sucked down the pump. The filter paper was removed by forceps to acined petri to which a drop of HCL was added and examined by stereoscopic microscope 3.5. objective 10 eye piece. *S. bovis* eggs stained brilliant pink to red and contrasted well with unstained faecal debris. The number of eggs per gram of faeces e.pg. were calculated according to the formula:

\[
e.p.g. = \frac{\text{No. of eggs counted in 3 filter papers} \times 35}{\text{weight of faeces} \times 3}
\]

2.6.3. Perfusion of adult worms from goat:

Nine goats exposed to different levels of *S. bovis* cercariae were perfused between 189-196 days of infection. Each goat prior 15 minutes to necropsy. The perfusion of goats was done according to the technique descried by Bushara (1978):

To induce hepatic shift, goats were injected with (10000-15000) i.u. of heparin to reduced blood clotting. The goat was slaughtered. The left fore and hind limbs were abducted carefully to avoid rupturing blood vessel. The skin of the thoracic and abdominal regions was deflayed and the ribs at the sternal junction are reflected. This exposed the thoracic and abdominal cavities so that any abnormalities could be noted. The thoracic aorta was carefully exposed and an incision was made into its lateral aspect at the level of the 6\(^{th}\) rib. An endo tracheal tube connected through a peristaltic pump to a reservoir containing
perfusion fluid was inserted through the incision towards the diaphragm and pushed posteriorly a distance just before the position of the coelioaco mesenteric artery. The tracheal tube was firmly secured inside the aorta by means of an inflation collar. The aorta was ligated by forceps immediately anterior to the opening. The accessory lobe of lung was reflected to expose the posterior venacava which was also ligated. An opening was made in the posterior dorsal part of the rumen avoiding blood vessels. Through this opening, ligated with a suture, and reflect until the right side was exposed and a pouch created in the greater omentum at the right oesophageal groove. Another pouch was made in the lesser omentum so that the pancreas was exposed and pushed aside to reveal the portal vein. The latter was opened in this region and any blood which collected in the pouch was removed by a rubber tube connected to a suction pump via collected vessel. Perfusion fluid (0.855% NaCl+0.05 sodium citrate) was pumped through the tracheal tube into the aorta first at low than at high pressure. Mean while the liver and intestines were constantly massaged. The perfusion containing adult worms passed through the opened portal vein into the abdominal pouches and was collected by the suction pump into the collection vessel. The contents of the latter were transferred into buckets and perfusion continued until the perfusate became clear. The fluid contents were allowed to settle and the supernatant discarded. The worms were then collected in small beakers and kept in saline and
placed in a refrigerator overnight. The worms were preserved in Ruda-Bush solution (distilled water (460cc), 95% ethyl alcohol(240cc) formalin (150cc) glycerine (100cc) and glacial acetic acid) and counted individually.

2.6.4. **Worm counts:**

The fluid was collected in big glass bottle the supernatant fluid remaining after sedimentation was poured through a screen to catch floating worms and then discarded. The last few liters of fluid and sediment were placed in 1 liter beakers, water being added at each step to haemolyse blood cells and dilute fine debris. The sediment was then transferred to petri dishes and the worm counted using a dissecting microscope.

2.6.5. **Tissue egg count:**

Tissue egg count was made according to Cheevers (1968) digestion method. Samples weighing 50 grams were taken from different parts of the liver, small and large intestine of each perfused animals. Samples were stored at-20°C till needed. For digestion; 25 grams of each sample were taken, sliced into smaller parts and placed in 250ml 5% KoH solution in a glass container and incubated at 37°C for 18 hours. The digest was then thoroughly agitated and three 1 ml samples were transferred to sedgwick Rafter counting chambers and the eggs counted microscopically. Tissue egg count per gram was calculated as follows:
No. of eggs count x volume of fluid
3
weight of samples

2.7. Histopathological methods:

Tissue samples for histopathology were taken from liver, lung, heart, spleen, pancreas, lymph nodes, small and large intestine. All tissues were fixed in 10% formal saline, transferred to automatic tissue processor and embedded in paraffin, cut at 5mm sections and stained with Haematoxylin and Eosin.

2.8. Statistical methods:

The experimental data obtained for 32 weeks has been subjected to statistical analysis.

The statistical analysis was performed using SPSS. Analysis of variance (ANOVA) test was carried out to examine the effect of different levels of *S. bovis* cercariae on the haematological values, parasitological finding and serum proteins. The results are presented as means±standard deviation (S.D.).
CHAPTER THREE
RESULTS

3.1. Clinical observations:

Infected animals showed no clear clinical signs of ill health during the first four weeks of infection except a temporary cough, particularly in animals of group (A). Infected animals of group (A and C) and (B) showed onset of diarrhoea the 6th week and 8th week respectively. Around the 9th week the mucohaemorrhagic diarrhoea became conspicuous in group (B) and around 7th week in group (A and C). This observed symptoms were continuous in group (B) and (C). All infected animals had dull appearance, general weakness, sunken eyes, particularly between the 2 month and 3 month from the infection and in this period they showed little interest in their food and the coat lacked luster particularly in group (C). In group (A) (500 cercariae /kg body weight) two animals (No. 27, 37) died after 6 weeks and 10 weeks respectively after infection and could not be perfuse. In group (B) (2000 cercariae/kg body weight) one animal died (No. 34) after three days of infection.

In group (C) (5000 cercariae /kg body weight) two animals died (No. 36, 41) after 9 and 10 weeks of experiment respectively and (No. 36) could be perfused. Also two goats of the control group (No. 39, 88) died suddenly on week 6 and week 9 of experiment respectively.
3.2. Body weight:

Mean weekly body weights for all experimental groups are presented in Fig. (1).

All the experimental goats gained weight steadily during the first four weeks before infection and the control continued to do so as the experiment progressed.

In group (A) (500 cercariae /kg body weight) this was followed by a gradual decrease from week four till week 19 of infection. At the end of the experiment, these animals lost an average of (1.4kg) from their starting body weights (from 15.4 to 14kg).

On the other hand, group (B) showed a decline during week three and four of infection. The starting weight values were lower than the end weight values from (16.4 to 17.88). Group (C) showed similar picture to group (A), body weight values declined at week eight and remained low (12.5kg) at week 17. At the end of the experiment these animals lost an average of (4.7kg) from their starting body weight (from 16.7 to 12kg).

Controls (group D) showed a gradual increase till the end of the experiment. At the end of the experiment these animals gained an average of (4.5kg) from the starting body weights (From 17.5 to 24 kg).
Fig1: Mean values of Body Weight in Experimental Goats.
It is evident that infected animals gained less weights than their control and the lowest body weights recorded were those for infected animal (group C) which were exposed to (5000 cercariae. kg body weight). Statistical techniques verified highly significant difference (P<0.001) between infected and control. The means of body weight values were significantly difference for group (A) (P<0.02) while group (B) and (C) recorded significantly lower means of body weight (P<0.01).

3.3. Haematological changes:

3.3.1. Haemoglobin concentrations Hb):

The mean haemoglobin concentrations are plotted in Fig. (3). In infected animals (group A, B and C) there were no striking changes in Hb concentrations during the first four weeks of infection. A gradual decrease was observed in the infected animals of group (C) from an average of about (7.9g/dL) of blood at the first week of the infection to (5.4g/dL) at week 14 of infection and the values were subsequently maintained lower than initial values till the end of the experiment (from 7.9 to 4). Infected animals of group (B) experienced a gradual fall in Hb concentrations, starting from (7.9g/dL) at the first week of the infection to (4.2/dL) at the 15 week of infection. This was followed by a gradual increase and the values remained high till the end of experiment. Haemoglobin concentrations in infected animals of group (A) showed a slight decrease from week two to week 14 but started to increase slightly till the end of the experiment.
Fig. 2: Mean Values Of Hemoglobin Concentration In Experimental Goats.

- GA (500 Cercaria)
- GB (2000 Cercaria)
- GC (5000 Cercaria)
- GD (Control)
Estimation of Hb concentration values in experimental goats showed highly significant difference (P<0.000) affected by the infection of *S. bovis* cercariae. Comparison of the Hb concentration values from the infected groups showed that group A and C recorded significantly lower means (P<0.05), while group B recorded significantly higher means of Hb concentration (P<0.05).

On the other hand, Hb concentrations in control animals (group D) slightly decreased during the first 7 weeks and remained higher than initial values till the end of experiment. Hb values in control animals were significantly higher than those of infected animals (P<0.05).

**3.3.2. Packed Cell Volume (PCV):**

The average PCV levels in experimental animals are presented in Fig. (4). Changes in PCV were similar to those described for haemoglobin in infected animals. Group (A) showed gradual decrease especially after the 9th, 12th and 15th weeks of infection. In the terminal weeks of the experiment there were a slight rise but lower than the initial values. Group (C), on the other hand, showed a sharp decrease at the second weeks of the infection and maintained low for the following 7 weeks and then increase for 3 weeks. The lower value was recorded at week 14.
Fig. 3: PCV Values in Experimental Goats

Weeks of Experiment

Packed Cell Volume

GA (500 Cercaria)
GB (2000 Cercaria)
GC (5000 Cercaria)
GD (Control)
Marked fluctuations were observed there after with smaller peaks recorded at weeks 16 and 24 in the terminal weeks of the experiment there were a sharp decrease in the PCV till the end of the experiment. Animals on the high dose of cercariae showed lower PCV percentages than the two other infected groups. The PCV values in the control group (group D) showed higher PCV percentages than those in infected animals.

Estimation of PCV in experimental goats showed that it was highly significant (P<0.001) affected by *S. bovis* cercariae. Comparison of the values from the infected groups showed that there were non-significant differences between the means of PCV values.

3.3.3. Total White Cell Counts (WBC):

Average WBC counts were presented in Fig. (5).

Infected animals of group (A) showed WBC counts that had an increase at the first week of infection till week 4, then decreased during weeks 5 and 6. From week 7 till week 13 the values were increased and then decrease at week 15. The values increased sat week 19 and 21. However, there was a slight rise during the terminal weeks of the experiment.

Infected animals of group (B) showed increasing counts from week 7 till the end of the experiment. Group (C) infected animals had slight rise at week 7 and the values dropped by week 10.
Fig. 4: Mean WBCs Counts In Experimental Goats.

- GA (500 Cercaria)
- GB (2000 Cercaria)
- GC (5000 Cercaria)
- GD (Control)
Thereafter the values fluctuating till week 14 and again slightly increased during the terminal weeks of the experiment. The first peak recorded at week 19-21 for 8 for group (A and C), at week 21 for group B. But the second peaks were recorded at week 13 for group (a) at week 15 for group B and at week 21 for group C.

Control animals (group D) showed wide fluctuations in WBC counts. They showed some increasing in WBC counts but these were comparatively minor. Statistical analysis revealed no significant differences (P>p.05) in WBC counts between the infected animal groups and control group.

3.4.3. Red Blood Cell Counts (RBCs):
Average weekly RBC counts can be seen in Fig.(6).

In infected animals of group (A) there were striking changes in RBC counts during the first 14 weeks of the infection. The lowest counts were reported at week 8-16 and 17. Also there were increased at week 22 till the end of the experiment. Group (B) showed decreasing during the 5-8 weeks of the infection. Then the counts slightly increased till week 9. This was followed by a slight gradual decrease till week 17 of infection. The count then slightly increased till the end of the experiment. Group (C) showed slight changes in RBC counts during the first 3 weeks of infection but a gradual decrease was observed during the following fourth week. This was followed by a slight gradual increase till week 13 of infection. The counts then decreased and another increased then remained till the end of the experiment.
Fig. 5: Mean RBCs Counts In Experimental Goats.
On the other hand, the control group showed less marked fluctuations in RBC picture although relatively higher values were observed at week 19-21 of the experiment (9.42x10^9/L). The lowest value (4.84x10^9/L) were observed at week 7.

The reduction patterns of RBCs count obtained from control and infected goats, when subjected to one way ANOVA and comparison of means via Scheffe test, indicated highly significant difference (P<0.01). Regarding the values of the means of the RBC count, it was found that there were non-significant differences between the infected groups.

3.3.5. The haematological indices:

Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) values were presented in Fig. 7, 8 and 9. Group (A) showed higher MCV values during the first 5-7 weeks of infection then decreased till week 14. Marked fluctuations were observed thereafter with smaller peaks recorded at weeks 16, 24 and 25. Group B showed higher MCV values during the first two weeks of infection. The smaller peaks recorded at weeks 7, 16, 22, 25, 26 and 36. However, in group (C) there was a slight rise in MCV values during the 5-6 weeks of infection. The smaller peaks recorded at weeks 14, 16, 17, 24 and 28.

Control animals (group D) showed no significant changes in MCV values throughout the experimental period except that the values remained slightly higher than the normal values during weeks 6, 15, and 20.
Fig. 6: Mean changes in MCV values in experimental goats

![Graph showing mean changes in MCV values over weeks of experiment for different groups: GA (500 Cercaria), GB (2000 Cercaria), GC (5000 Cercaria), and GD (Control).]
Fig. 7: Mean changes in MCH experimental goats

Weeks of experiment

MCH / Ppg

GA (500 Cercaria)
GB (2000 Cercaria)
GC (5000 Cercaria)
GD (Control)
Fig. 8: Mean changes in MCHC values in experimental goats
Estimation of MCH in the experimental goats showed that it was highly significant (P<0.01) affected by the infection of *S. bovis* cercariae. When the values for the infected groups were compared, they showed non-significant differences (P>0.01).

Estimation of MCH and MCHC in the experimental goats showed that there were non-significantly affected by the infection of *S. bovis* cercariae.

### 3.4. Biochemical parameters:

#### 3.4.1. Serum proteins:

##### 3.4.1.1. Total proteins:

Total protein values are presented in Fig. (10).

Total protein showed little changes throughout the experimental period with a slight tendency to increase. However, in group (C) the total protein tended to be high at weeks 3, 4, 13, 15 and 16 and 20 of infection, while in group (B) a slight increase was observed at weeks 10, 11, 13, 16, 19 of infection then returned to normal levels. There after the values were fluctuating and a high value were recovered at weeks 21 of infection.

Control animals (group D) showed little changes throughout the experimental period showed that there were non-significant (P>0.05) difference between the infected groups and control.
Fig. 9: Total protein levels of normal and *Schistosoma bovis* infected goats.

[Graph showing total protein levels over weeks after infection for different groups: GA (500 Cercaria), GB (2000 Cercaria), GC (5000 Cercaria), GD (Control).]
3.4.1.2. Serum Albumin:

Mean weekly serum albumin for all experimental groups are presented in Fig. (11).

Serum albumin, on the other hand, was slightly elevated in infected animals, with minimum values (2.62g/L) for group (A), (30g/L) for group (B) and (21.7g/L) for group (C) at the 9th weeks of infection respectively.

The values for control animals ranged between (30.7-50.2 g/L).

Measurement of experimental goats serum albumin concentration showed that its values were non significantly (P>0.05) affected by the infection *S. bovis* cercariae.

3.4.1.3. Serum globulin

Serum globulin values of infected animals ranged between (78.2-31.9g/L) for group (A), (83.5-22.7) from group B (107-30) for group (C), while the control (group D) ranged between (82.4-22.0). Measurement of experimental goat’s serum globulin concentration showed that its values were non significantly (P>0.05) affected by the infection *S. bovis* cercariae the results were shown in Fig. 12.

3.4.1.4. Albumin/globulin ratio:

The A/G of uninfected animals group D ranged between (1.43-0.45g/L) while the range of infected animals of group (A) was (1.68-0.45g/L), group (B) was (1.62-0.51g/L) and group (C) was (1.75-0.31g/L). The results were shown in Fig. 13.
Fig. 10: Albumin levels of normal and *Schistosoma bovis* infected goats

![Graph showing albumin levels over 22 weeks after infection for different groups: GA (500 Cercaria), GB (2000 Cercaria), GC (5000 Cercaria), and GD (Control).]
Fig. 11: Globulin levels of normal and *Schistosoma bovis* infected goats

![Graph showing globulin levels over weeks after infection for different infective doses.](image-url)
Fig. 12: Albumin Globulin Ratio of normal and *Schistosoma bovis* infected goats.
3.5. Parasitologicl finding:

3.5.1. Faecal egg counts:

Eggs were first appeared in the faeces of group (A and C) at week 6 and group (B) at week 8 of the infection.

Group (A) showed that the number of eggs increased at week 9 to week 13 and then increased to reach maximum number of eggs at 14 and week 15 then decreased till the end of the experiment. Group (B) showed that the number of eggs increased till it reached 12 weeks of infection then fluctuating till it reached the maximum number of eggs at week 18. The number of eggs after that the number of eggs increasing from week 7 and reached the maximum value at week 18 then decreasing from week 23 till the end of experiment. Comparison of the values from the infected groups showed that there were highly significant (P<0.01) differences between the groups. The results were shown in Fig. 14. shown the parameters values of infected groups at maximum values of faecal egg counts.

3.5.2. Worm recovery:

The results of worm recovery in goats infected with *S. bovis* cercariae were shown in (Table 1). An attempt was made to differentiate male and female worms recovered from group A, B and (C). The percent of recovery of infected goats were higher in group (C) than the two other group.
Table 1: Experimental design and worm recovery in *Schistosoma bovis* infected goats.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Goat No.</th>
<th>Infective doses (S. bovis cercaria)</th>
<th>Male</th>
<th>Female</th>
<th>Worm pairs</th>
<th>No. of worm recovered</th>
<th>Mean recovery</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>12</td>
<td>500</td>
<td>88</td>
<td>2</td>
<td>10</td>
<td>110</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>500</td>
<td>41</td>
<td>6</td>
<td>0</td>
<td>47</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>500</td>
<td>100</td>
<td>6</td>
<td>0</td>
<td>106</td>
<td>60.2</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>500</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>500</td>
<td>25</td>
<td>1</td>
<td>0</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>19</td>
<td>2000</td>
<td>211</td>
<td>25</td>
<td>36</td>
<td>308</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2000</td>
<td>56</td>
<td>2</td>
<td>0</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>2000</td>
<td>N.D</td>
<td>N.D</td>
<td>N.D</td>
<td>N.D</td>
<td>103.75</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>2000</td>
<td>44</td>
<td>1</td>
<td>2</td>
<td>49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>36</td>
<td>5000</td>
<td>515</td>
<td>36</td>
<td>41</td>
<td>633</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>5000</td>
<td>420</td>
<td>27</td>
<td>25</td>
<td>497</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>5000</td>
<td>519</td>
<td>139</td>
<td>22</td>
<td>702</td>
<td>697</td>
<td>13.9</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5000</td>
<td>660</td>
<td>110</td>
<td>93</td>
<td>956</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N.D.= not done

Fig. 13: Feacal egg count following infection with *Schistosoma bovis*
Fig. 14: Tissue egg counts of group A, B, C, and D after infected with 500, 2000, and 5000 Schistosoma bovis cercaria per kg body weight respectively.
3.5.3. Tissue egg counts:

The results were shown in Fig. (16). The analysis of the results of tissue egg counts in goats infected with *S. bovis* and the mean values of tissue egg counts of group (A, B and C) of infected goats were shown in Table (2, 3) respectively.

Goats of group (C) produced the highest tissue egg counts in the liver, small and large intestine. The goats (No. 12) and No. (35) of group (A) and (B) respectively did not exhibit any tissue egg counts in the liver.
Tables 2: Tissue egg counts of group A, B. and C of goats after infection with 500, 2000 and 5000 *Schistosoma bovis* cercariae per kg body weight respectively.

<table>
<thead>
<tr>
<th>Group of animal</th>
<th>Goat no.</th>
<th>e.p.g.</th>
<th>Total tissue eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Group A</td>
<td>12</td>
<td>Zero</td>
<td>613</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>65</td>
<td>260</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>20</td>
<td>515</td>
</tr>
<tr>
<td>Group B</td>
<td>19</td>
<td>59</td>
<td>6560</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>70</td>
<td>1840</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>Zero</td>
<td>1150</td>
</tr>
<tr>
<td>Group C</td>
<td>38</td>
<td>177</td>
<td>15060</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>195</td>
<td>15270</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
<td>14280</td>
</tr>
</tbody>
</table>
Table 3: the means of tissue egg counts of group A, B and C of goats infected with 50-0, 2000 and 5000 *Schistosoma bovis* cercariae per kg body weight respectively.

<table>
<thead>
<tr>
<th>Group of animal</th>
<th>Mean ± SD</th>
<th>Mean total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Group A</td>
<td>28.3±33.291</td>
<td>462.66±182.2</td>
</tr>
<tr>
<td>Group B</td>
<td>43±37.643</td>
<td>3183.3±2944.5</td>
</tr>
<tr>
<td>Group C</td>
<td>157.3±50.461</td>
<td>14870±521.63</td>
</tr>
</tbody>
</table>
3.6. Pathological changes:

3.6.1. Macroscopic findings:

Varying degree of emaciation and loss of body fat, was observed in infected animals particularly those of group (C). The liver showed small whitish grey foci diffusely scattered on the surface, especially group (A). Various degree of colour changes were seen in the lungs of all infected animals. All groups showed changes colour of kidney, which were otherwise grossly normal. The small and large intestine showed diffuse hyuperaemia, and mucosal haemorrhages and the intestinal contents were watery and haemorrhagic, particularly those of group C.

3.6.2. Microscopic findings:

Histologically the most prominent lesions were parasite granulomatous reaction associated with the location of the parasite and/or their fragments in the blood vessels and tissue of lung, liver, pancreas, intestine and mesenteric lymph node. These lesions were frequently encountered in tissue of animal of group C followed by group B and were scarcely detected in tissue of animal of group A. However, the intensity of lesions in the intestine and mesenteric lymph nodes were similar in all groups, whereas lesions in pancreas were presented by group C only. In advanced parasite disintegration cavities were formed. Blood vessels revealed phlebitis evidenced by intimately proliferation, degeneration and fragmentation (Plate 3).
**Plate 3**: Animal Gr. A. Liver: Note proliferation and fragmentation of blood vessels, ova granuloma cavitations. H & E × 10

**Plate 4**: Animal Gr. A. Liver: Note hepatocellular swelling and vacuolation. H & E × 10.
The inflammatory exudates of the granuloma were fairly consistent and consisted of mononuclear cells mainly lymphocytes, epithelioids and proliferation of fibrous tissue. Intense effusion of polymorphonuclear cells were frequently recognized in tissue of group C, while giant cells only seen in lymph nodes of group A. Eosinophils were scarcely observed and when present were detected in areas devoid of parasites or their fragments.

3.6.2.1. Liver:

Animals of group C: The most prominent lesion included hepatocellular swelling, vacuolation (Plate 4), ova granuloma and intense infiltration of mononuclear and polymorph nuclear cells in the portal tract (Plate 5). However, the mononuclear cells were more predominant in-group (A) whereas the polymorphs were predominant in animals of group C. Furthermore, most of the animals of group A revealed haemorrhages and granuloma with cavity formation.

3.6.2.2. Lung:

Lung of animal of group A, B and C showed oedema and focal areas of interstitial pneumonia evidenced by the changing of alveolar septa with thickening and proliferation of fibroblast and effusion of mononuclear cells, mainly lymphocytes (Plate 6). The reaction of group A and B was mild, where as in group C the pneumonic areas were more marked and presented ova granuloma (Plate 7). However, schistosoma parasite, which was devoided of inflammatory exudates, was present in blood vessels of lung of group A (Plate 8).
**Plate 5**: Animal Gr. A. Liver, Note intense lymphoid cell infiltration in portal tract around degenerated ova. H & E × 10.

**Plate 6**: Animal Gr. C. Lung: Note thickening of alveolar septa H & E × 10.

3.6.2.3. **Kidney:**

All groups showed glomerulo-interstitial nephritis. However, kidney of animal of group B and C revealed milder changes comparable to those of group (A), which presented severe reaction. In the latter the glomeruli, and tubular epithelium showed necrosis, fragmentation and intense infiltration of lymphoid cells in the connective tissue. In some areas, the kidney tissue was obliterated and replaced by inflammatory exudates (Plate 9). Kidney of animal of group C, presented homogenous pink hyaline deposits in Bowman’ space (Plate 10).

3.6.2.4. **Heart:**

All groups showed myocarditis with effusion of aggregates of lymphoid cells (Plate 11), only animal of group A showed vacuolation of myocardial cells (Plate 12).

3.6.2.5. **Pancrease:**

Only animal No. 58 group (C) showed acinar atrophy, ova granuloma concomitant with intense infiltration of lymphoid cells around the ova granuloma and the interacinar spaces (Plate 13).

3.6.2.6. **Spleen:**

All groups showed haemosidern pigments (Plate 14).

H & E ×10.


3.6.2.7. Lymph node:

All groups showed similar changes including, ova granuloma, which is more prominent in cortex, oedema and intense infiltration of mononuclear cells which, consisted of lymphoid cells, plasma and macrophage, in the sinuses. Occasionally, giant cells areas encountered in lymph nodes of group A only. (Plate 15, 16 and 17). Haemosiderin and haemosiderin laden macrophage were frequently observed in sections of lymph nodes of group (C). Parts of the schistosoma parasites were seen in serosa and tabiculus vessels. The presence of the parasite in these areas was accompanied by infiltration lymphoid cells and eosinophils.

3.6.2.8. Intestine:

All animals in (C) group showed denuolation of superficial layer oedema, and ova granuloma associated with the accumulation of inflammatory exudates in the connective tissue, lamina propria submucosa and occasionally muscular mucosa. these cells were composed of lymphoid cells and some eosinophils. The ova were present in all layers of intestine. However, in animal of group A (Plate 18) the reaction was advanced with cavity formation compared to those of group B (Plate 19) and group C (Plate 20).


Plate 19: Animal Gr. B. Intestine: Note ova encompassed with accumulation of lymphoid cells. H & E ×10

CHAPTER FOUR
DISCUSSION

In the Sudan, animals are mainly kept under nomadic conditions, making seasonal migration in search for food and water. In the dry summer season animals crowd in large numbers around available water sites where conditions are conducive of infection of both the snail intermediate host and the mammalian host. High infection rates with *S. bovis* have been reported in sheep and cattle during this period (Majid *et al.*, 1980a; 1983). The disease in animals due to *S. bovis* is considered to be of economic importance and outbreaks resulting in high mortalities and loss of revenue have been reported in various parts of the world (Veglia and Leroux, 1929; Strydom, 1963; Dinnik and Dinnik, 1965; Eisa, 1966; Hurter and Potgieter, 1967; Hussein, 1968; Reinecke, 1970). In the Sudan, *S. bovis* is endemic along the White Nile, in Singa and Gedarif region, the Gezira, Ingasana area, in the western state Province of Kordofan, in Darfur, and in the southern States of Bahr-el-Ghazal. Natural infections were reported in cattle, sheep, horses, donkeys and camels (Malek, 1969). The first reported outbreak of bovine schistosomiasis occurred in 1964 at Kosti district, in the White Nile Province (Eisa, 1966) and in 1982, another outbreak occurred in sheep in Darfur Province (Majid, Unpublished observation) killing thousands of animals.
Although excellent studies have been carried out on the immunological, pathophysiological, pathogenesis and epizootological aspects of schistosomiasis in domestic livestock in the Sudan (Hussein et al. (1980), Saad (1979), very little work had been carried out in sheep (Majid et al., 1983) and even less so in goats.

Therefore, the present study was conducted to investigate the clinical and pathological alteration imposed by S. bovis on goats. The objective of such work is to reveal the difference between infected groups at high, medium and low dose of S. bovis cercariae infection.

The results of the present work confirmed the susceptibility of the male Nubian goat to experimental infection with S. bovis, which has been previously established.

This study has shown that goats infected with low (500 cercariae/kg), medium (2000 cercariae/kg) and massive doses (5000 cercariae/kg) of Schistosoma bovis first became clinically ill around 6th week in group (A and C) and at 8th week in group (B) of infection and during the following 9th week the mucohaemorrhagic diarrhoea became conspicuous in group (B) and around the 7th week in group (A and C). During the following 2-3 months all infected groups became progressively anaemic, hypoalbuminaemic and hyperglobulinaemic, developed a marked increase in total white cell and either lost weight or failed to maintain the rate of growth recorded in uninfected animals. These
clinical observation of the animals were related to the first appearance of eggs in the faces and were maximal at the time when faecal egg count was highest. These changes have all been reported previously in cattle infected with *S. bovis* or *S. mattheei* (Preston *et al.*, 1973a). All of these changes were strongly related to the level of infection.

The infected goats showed a decrease in their body weight as compared with the control. There is no doubt that goats of group (C) suffered greater weight loss and this was evident as early as the 4th week of infection and was more pronounced form week 12 till the end of the experiment. This study also showed that at the maximum body weight value (24.33kg) of control group (D) the body weight of infected groups was 12.67kg for group (A and C) and 17.75kg for group (B). Similar loss of the body weight was recorded in calves infected with *S. bovis* (Saad *et al.*, 1984). Osman (1984) found that sheep infected with 4000 *S. bovis* cercariae had reduced body weight gain as early as the end of infection and by 16th week the poorly fed infected animal lost 3.7 kg compared to 1.42 kg in their better fed counterparts. Saad (1979) found that *S. bovis* adversely affected the growth rates of the calves. This became first apparent around the time of patency, was particularly marked between the second and fifth months and found that *S. bovis* adversely affected the growth rates of the calves. This became first around the time of patency, was particularly marked between the second and fifth months and was
related to the level of infection. Dargie and Berry (1979) observed that acutely infected livestock lost up to 7kg/week whereas their chronically infected counterparts gained 1.0 to 2.5 kg less weight/week than do uninfected animals of the same initial bodyweight. Those authors attributed this depletion to the general behaviour of the infected goats which suffered from a degree of inappetance during the acute stage of the disease and in addition to the protein intake and dehydration (Berry and Dargie, 1978 and Preston et al., 1973a). Since *S. bovis* mainly involves the liver and intestinal tract other factors such as impaired liver function or disturbed digestion and absorption of dietary nutrients maybe involved (Berrh et al., 1973; Saad et al., 1980). Diarrhoea and loss of blood through intestinal haemorrhage may also be contributing factors. The lesions due to schistosome eggs were located mainly in the mucous and the submucous layer overlaying the submucous vascular arcades. Granulomas destroyed ganglia, nerve fibre strands and nerve fibre. These changes imply reduced functional capacity in the nervous tissue, which might cause reduced motility, mal absorption and partly account for the loss of body weight.

The present results showed that infected animals gradually developed anaemia starting from the 4th week of exposure: Hb, PCV and RBC counts decreased markedly especially between week, 6 and 10, and group (C) recorded the lowest values. Control goats showed no significant change, and group (A) had
consistently higher value. No appreciable variation between control and infected animals in the haematological indices MCV, HCH and MCHC were recorded and this may be due to that the anaemia produced is of normocytic normochromic type. However, lower Hb, MCV and MCHC values have been reported by Gameel (1974) in sheep naturally infected with \textit{S. bovis} which suggest the occurrence of mild normocytic hypochromic anaemia. Reduction in red cell mass was also reported in experimental \textit{S. matteei} infection in sheep (Preston \textit{et al.}, 1973a).

The studies of Dargie \textit{et al.} (1973), and Preston and Dargie (1974) in sheep infected with \textit{S. matteei} indicate that anaemia in schistosomiasis is principally haemorrhagic, resulting from loss of blood elements and plasma proteins from the gut. Similarly, Saad \textit{et al.} (1980) described anaemia in calves infected with \textit{S. bovis} between the 5\textsuperscript{th} and 12\textsuperscript{th} weeks of infection, which coincided with the time of appearance of eggs in faeces and was maximal, when faecal egg was highest. Schistosomal anaemia may also be associated with haemodilution (Preston and Dargie, 1974). Dargie (1980), Markoto (1931) observed that rabbits infected \textit{with} \textit{S. japonicum} developed anaemia before appearance of other clinical symptoms and related that to the production of toxins by the parasites or the eggs. Smith and Jones (1966) reported that schistosomiasis may cause anaemia through haemorrhages caused by mechanical action of ova or by miracidial enzymes.
All infected goats of the current study showed high WBC counts during the different period of the experiment according to the level of infection. No attempt was made to calculate the different types of leukocytes in the present study. However, higher values of white blood cells count were recorded at week 11 till the end of experiment for group (A and B) and at week 20 till the end of the experiment. Lengy (1962b) reported that the increase of WBC was mainly due to a corresponding increase in neutrophil and eosinophil counts and seems to coincides with the start of patency. He also detected eosinophilia in sheep with heavy S. bovis infection but this was noticed 4 weeks after exposure. Gameel (1974) reported high WBC neutrophil counts in Sudanese sheep naturally infected with S. bovis but he could not detect any changes in eosinophils. Saad et al. (1980) reported eosinophilia in calves 3-4 weeks after exposure with maximum values recorded at week 8 and this was higher in heavily infected animals.

The present study revealed that the values of total protein and its fractions, albumin and globulin in between the infected groups (A, B and C) are not significantly changed. However, there was an increase in total protein level during the experiment in all infected groups compared with the control group (D). Also there was slight increase in albumin level during the experiment in all infected groups compared with control group. This result agreed generally with that reported by (Endrejat, 1956; Shumard et al., 1957; Kuttler and Marble, 1960; Tuner and Wilson, 1962;
Wilson and Turner, 1965; Dobson, 1966), that the occurrence of hypoalbuminaemia accompanied by hyperproteinaemia seemed to be the pattern in helminthic infections. Saad (1979), using radioisotopic tracer technique, showed that in cattle and sheep experimentally infected with *S. bovis* there was hypoalbuminaemia due to an increased rate of albumin catabolism with marked depletion of albumin particularly from the extra vascular pool.

Similarly hypoalbuminaemia was recorded in calves infected with *S. bovis* (Saad, et al., 1984). Those authors attributed this depletion to hypercatabolism and plasma volume expansion, which in variably occurs with disturbances in plasma protein concentration. This relation between the depletion of body albumin and the hypercatabolism was reported to be associated with loss of albumin into the gastrointestinal tract (Dargie et al., 1973). Hypoalbuminaemia reported in Zebu cattle exposed to very heavy infection of *S. bovis* was attributed to the increased red cell losses and higher rates of albumin catabolism (Bushara et al., 1980).

In the current study there is no statistical significant effect within infected groups these may be attributed to the short period of experiment. The present study revealed that the prepatent period was 42-56 days while Massoud (1973) reported that it was 47-48 days in goats.
The faecal egg count of the current study showed statistical differences between the infected groups and this was related to the level of infection. That means the eggs passed in the feces correlated with the intensity of the infection. The data also showed a gradual increase in faecal egg count with the time of infection, till it reached a maximum value then a gradual decrease to minimum value. Faecal egg count may be regarded as a quantitative index of pathogenicity: for example counts of *S. matteei* in sheep above 50 eggs/gm are indicative of economically significant levels of parasitism (Lawrence, 1977). It appears from the present finding that maximum egg count occurs between 10-12 weeks of infection for all infected groups.

In the current study the highest total worm recovery was 13.9% for group C, followed by 11.6% for group A and 6.8% for group (B), compared with a rate of 24% and 26% as described by Elsamani (1999) and Adam and Magzoub (1977). In calves experimentally infected with *S. mnsoni* recovery was 1.2% (Saeed *et al.*, 1969). These authors suggested that the low rate of worm recovery probably under estimate the real take of infective cercariae. This suggestion may correspond to the present work since it was found earlier by Clegg and Smith (1978) that a constant percentage of cercariae depending on the animal species, die within skin during penetration. Also the structure and composition of the skin are involved in decreasing the number of penetrating cercariae.
The recovery of female worms was very low about 7 times less than the male. This differ from that of females recovered from calves experimentally infected with *S. mansoni* (41-45%) and of females recovered from calves experimentally infected with *S. mattheci* (approximately 50%). The male /female ratio was (1:7) while that of the work done by Adam and Magzoub (1977) was 1/0.1 and Elsamani (1999) was 1/0.7.

The low percentage of excreted eggs and of eggs per gram counted in tissues may be attributed to the low percentage of cercariae which later give female worms as compared to that of males. This may be due to the fact that the experimental infection where the repeated exposure of the animals to large numbers of infective stages give the chance to both sexes to infect these animals.

This study showed that the total number of eggs in the tissues was proportional with intensity of infection and the small intestines being the predominant site of oviposition in all infected groups. This may be due to the migration of eggs to the small intestines after 6 month of infection. The low number of eggs detected in the liver may be due to the destruction of eggs by the granulomatous reactions.

The gross lesions observed especially in the liver were typical of ovine schistosomiasis and were consistent with the findings of Hussein *et al.* (1976) and Saad *et al.* (1984). However, the above authors reported pronounced changes in the different
organs examined and this seems to be related to the high infection
doses. Hydroperitonium was recorded to be a prominent feature
of ovine schistosomiasis (Hussein et al., 1976) and this has also
been reported in sheep infected with S. mattheei (McCully and

The histopathologicl lesions observed in this study were in
general agreement with those previously reported in S. bovis
infection in sheep (Gameel, 1974; Hussein et al., 1976; Saad et
al., 1984) and cattle (Hussein, 1968, 1971; Hussein et al., 1975)

In the present study the severity of the lesions in group (C)
was greater than the group (B) and (A). However, the intensity of
lesions in the intestine and mesenteric lymph nodes were similar
in all groups, whereas lesions in the pancreas were presented in
group (C) only.

Despite the observed pathological changes in the different
tissues of the infected animals, the main disease syndrome was
basically due to the excretion of eggs into the alimentary tract.
This confirms the findings of earlier workers (Saad et al., 1980)
that the pathogenesis of ruminant schistosomiasis was due mainly
to the egg excretion rather than to egg retention in the different
tissues. This explains the fact that clinical alterations coincided
with the onset of oviposition and are maximal when the faecal egg
counts are highest.
للفجوات والامعة واللممة والعقد في حبيبة أوام ووجود إضافًا لحظ وقعد في الكلي فية التهاب وجود في اليموسرين صباغات وجدت في المرضية الأنسجة هذه وقعد القلب عضلات في التهاب أكثر وقوع في وضوح الحيوانات الأنسجة موعدة...
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