بسم الله الرحمن الرحيم

Sudan University of Science & Technology
College of Graduate Studies

Prevalence and Risk factors of Bovine Paramphistomiasis in Khartoum Bahri locality, Khartoum State, Sudan

نسبة الإصابة وعوامل الخطر لمرض دودة الكرش فى الأبقار بمحلية الخرطوم بحرى ,ولاية الخرطوم ,السودان

By
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قال تعالى :

بِسمِ اللهِ الرَحمنِ الرَحِيمِ

(وَمِن الأَنعَامِ حَمُولَةً وَفَرشًا كُلُوا مِمَا رَزَقَكُمُ اللهُ وَلا تَتَبِعُواْ خُطُوَاتِ الشَيطَانِ إِنَهُ لَكُم عَدُوٌ مُبِينٌ)

صدق الله العظيم

سورة الأنعام الآية رقم (142)

Dedication

To the soul of my father

To my mother

To my daughter and my son

To my husband

Acknowledgments

Firstly and eventually great thanks for Got who bless and completing this work and I hope success in the future studies.

My appreciation and my thanks to my supervisor, Professor Galal Eldin Elazhari Mohammed Elhassan for his advices on continuous reviewing the dissertation.

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Abstract

A cross-sectional study was carried out on 330 of cattle in Kadaru slaughter house in Khartoum state, Sudan, during winter (December 2014 and January 2015). The objectives of study were to estimate the prevalence of paramphistomiasis in cattle and to investigate the potential risk factors associated with the disease.

The overall of cattle prevalence was found to be 12.7% when tested by fecal sedimentation test. The prevalence of the infection according to the age was 11% in animals equal and less than two years and 13.3% more than two years. The prevalence according to the sex was 33.3% female and 11.3% male. The prevalence according to the breed of the animals was 12.2% for local and 16.7% for cross. The prevalence according to the body condition was 12.1% for good condition and 42.9% for bad condition. The prevalence according to the source of the animals was 12.7% from Niala and 12.9% from East States and the prevalence according to the Fasciola infection was 66.7%.

Univariate analysis using the Chi-square, with confidence intervals of 95% at a *p-value* ≤0.25 was used to identify potential risk factors associated with fecal sedimentation test- positivity for paramphistomiasis infection in bovine. Significant positive risk factors associated with fecal sedimentation in the univariate analysis, there were found to be ***sex*** *(x^2 = 8.573, p-value = 0.003)*, **body condition***(x^2 = 5.845, p-value = 0.016)*, **Fasciola***(x^2 = 7.930, p-value = 0.005)*. There were also significant risk factors associated with fecal sedimentation positive in the multivariate analysis.

The multivariate analysis, using logistic regression, with a confidence intervals 95% *p-value* 0.05 was used to assess the association between identified significant risk factors in the univariate analysis in a combination towards a positive fecal sedimentation test status for

paramphistomiasis infection in bovine, the analysis showed association between the paramphistomiasis infection in bovine and sex (**Exp (B) = 3.627)** that means the infection in females equal **3.627** times in males, in body condition (**Exp (B) = 1.057)** that means the infection in bad condition equal 1.057 times in a good condition and fasciola (**Exp (B) = 0.084)** that means the infection in positive cases of fasciola equal 0.084 times in negative cases of the fasciola .

It could be concluded that the potential risk factors (sex, body condition and fasciola) were showed highly significant association with paramphistomiasis infection.

ملخص الدراسة

اجريت دراسة مقطعية لـــ 330 رأس من الأبقار فى ولاية الخرطوم محلية الخرطوم بحرى مسلخ الكدرو, خلال فصل الشتاء إعتباراً من الأول من ديسمبر 2014 وحتى 31 يناير 2015, كان الهدف من هذه الدراسة هو تقدير معدل إنتشار مرض دودة الكرش فى الأبقار والتحقق من عوامل الخطر المرتبطة بإنتشار مرض دودة الكرش.

كان معدل إنتشار المرض فى كل الحيوانات التى تم فحصها بإختبار ترسيب البراز هو 12.7%. كان معدل إنتشار العدوى وفقاً لسن الماشية 11% فى الحيوانات الأقل أو تساوى من سنتين و 13.3% للحيوانات الأكثر من سنتين. وكان معدل الإنتشار وفقاً لجنس الحيوان 33.3% فى الإناث و11.3% فى الذكور, ووفقاً لسلالة الحيوان كان معدل الإنتشار 12.2% للسلالة المحلية و16.7% للسلالة المهجنة , أما بالنسبة لحالة الجسم فكان معدل الإنتشار 12.1% للحالات الجيدة و42.9% للحالات الغير جيدة , وكان معدل الإنتشار وفقاً للمناطق التى جاءت منها الحيوانات 12.7% من نيالا و 12.9% من الشرق (ولايات الشرق) , ووفقاً للإصابة بالفاشيولا كان معدل الإنتشار 66.7%.

تم التحقق من عوامل الخطر الإيجابية المرتبطة بالمرض بإستخدام مربع كاى للتحليل $p\text{-value} \leq 0.25$ قى التحليل وحيد المتغير حيث كانت عوامل الخطر المرتبطة بإنتشار المرض هى:

جنس الحيوان $(x^2 = 8.573, p\text{-value} = 0.003)$, حالة الجسم $(x^2 = 5.845, p\text{-value} = 0.016)$

الإصابة بالفاشيولا $(x^2 = 7.930, p\text{-value} = 0.005)$

بإستخدام التحليل بالإنحدار اللوجستى $p\text{-value} \leq 0.05$ لمعرفة درجة الإرتباط بين إنتشار المرض وعوامل الخطر , أظهرت النتائج وجود إرتباط إيجابى بين مرض دودة الكرش فى حالة جنس الحيوان يكون معدل الإنتشار يساوى 3.627 أضعاف فى الإناث عنه فى الذكور **(Exp (B) = 3.627)** , وفى حالة الجسم يكون معدل الإنتشار يساوى 1.057 فى حالة الجسم غير الجيدة عنه فى الحالة الجيدة **(Exp (B) = 1.057)** وفى حالة الإصابة بالفاشيولا يكون معدل إنتشار المرض يساوى 0.057 فى الحالات الموجبة للفاشيولا عنها للحالات السالبة **(Exp (B) = 0.084)**.

أظهرت هذه الدراسة وجود إرتباط وثيق بين معدل إنتشار دودة الكرش فى الأبقار و جنس الحيوان وحالة الجسم و الإصابة بالفاشيولا.

Introduction

Paramphistomum is one of the common parasites in the rumen and reticulum of sheep, goats, cattle and water buffaloes. Light infection doesn't cause serious damage to the animals, but massive number of immature *Paramphistomum* can migrate through intestinal tract causing acute parasitic gastroenteritis with high morbidity and mortality rates, particularly in young animals. *Paramphistomum* parasite in duodenum and ileum are plug feeders and cause haemorrhage which leads to bleeding and diarrhoea, bleeding for prolonged period may cause anemia, which further weaknesses the host. Mature *Paramphistomum* are also responsible for ruminitis irregular rumination, lower nutrition conversion and loss of body condition, decrease in milk production and reduction of fertility (Mogdy *et al.,* 2009). *Paramphistomiasis* is worldwide in distribution, but the highest prevalence has been reported in tropical and sub tropical regions, particularly in Africa, Asia, Australia, Eastern Europe and Russia. The epidemiology of *Paramphistomum* is determined by several factors governed by parasite-host-environment interactions. The major epidemiological variable influencing worm burdens of animals is the infection rate from pastures It is also influenced by the climatic requirement for egg hatching, development and survival of the larvae in pasture (Melaku *et al*., 2012). Acute *paramphistomiasis* is caused by massive infection with immature worms in the small intestine. They attach themselves to the intestinal mucosa, drawing pieces of the mucosa into their suckers causing strangulation, necrosis and hemorrhage. Acute *paramphistomiasis* usually occurs in young cattle less than two years of age and is characterized by listlessness and anorexia. Profuse diarrhea (which can sometimes be projectile) develops two-four weeks after infection. The feces are very fluid and may even contain immature flukes. Sub-mandibular edema has been noted in several outbreaks and anemia

has also frequently been described. The association between the presence of adult flukes in the rumen and clinical disease has not been well established, although the presence of the parasite is often complicated by other concomitant conditions (associated with animals in poor condition, ill thrift and other parasitic diseases) (Waal, T. D., 2011). There is little evidence regarding the pathogenesis of adult flukes to their hosts, but severe damage to the mucosa of the rumen was reported in heavy infection in experimentally infected sheep (Eslami *et al*., 2012).

2. Synonyms:

Paramphistoma amphistomiasis

3. Justification:

paramphistoma infestation is an important disease but neglected and it is a public health problem in Africa, especially in rural communities . *Pramphistoma* is considered a major public health problem in Sudan (F.A.O.2009). Many animals are infected with *pramphistoma*. Determination of the prevalence of the disease in Khartoum state is important in order to explore the size of the problem which helps to control the disease. *Paramphistomiasis* infection is thought to be associated with the presence or absence of intermediate snail habitats in the grazing areas of the animals. Since tropical *paramphistoma* is a significant factor in limiting livestock production, the development of sustainable strategies for controlling *paramphistoma* infection is a priority. Strategic use of antihelmintics, enhancement of host resistance by genetic improvement, biological control and better herd management all have a role to play in sustainable control of *paramphistoma* (Eslami *et al*.,2011).

4. Objectives:

The objectives of this study were:

1. To estimate the prevalence of bovine *paramphistomiasis* in Sudan (Khartoum State).
2. To investigate the potential risk factors associated with the disease.

Chapter One

Literature Review

1. History:

The first report of the causative agent of *paramphistomatosis* of cattle was in the coastal region of Algeria is described. On the basis of histological finding, causative agent was found to be a *Paramphistomum. Daubneyi, Lymnaea Truncatula* was found to be present as a potential intermediate host at the localities where the cattle harboring these trematodes were kept. It was suggested that high intensity of invasion by the trematodes (up to 2204 specimens in one host) caused severe helminthosis in some cases. The finding of the causative agent of bovine *paramphistomatosis* in Algeria draws attention to the need of further investigation of trematodosis in cattle kept in that country (Pacenovsky *et al*., 1987).

2. LIFE CYCLE:

Paramphistomum parasite has an indirect life cycle with fresh water snails as the intermediate hosts, e.g. the genus Bulinus, Planorbis, Stagnicola,

These snails are found in permanent and temporary watercourses, irrigation channels, swamps, dam edges and depressions, they are normally found attached to vegetation in these habitats (NSW DPI 2007). Adult flukes in the stomach lay eggs that are shed outside with the feces. About 2 weeks later miracidia hatch out of the eggs. They swim in the water until they find a suitable snail. They penetrate into the snail and continue development to sporocysts and rediae, which can multiply asexually and produce daughter rediae. Each redia produces several cercariae, the next developmental stage. Out of a single miracidium up to 30 cercariae can develop. Cercariae abandon the snail, swim around and

attach to the vegetation where they encyst and become metacercariae, which are infective for final hosts that feed on infested vegetation. Encysted metacercariae do not survive dryness, but can survive and remain infective for up to 1 year in a humid.

Figure 1: Planorbid snails, the intermediate host for stomach fluke (NSW DPI 2007)

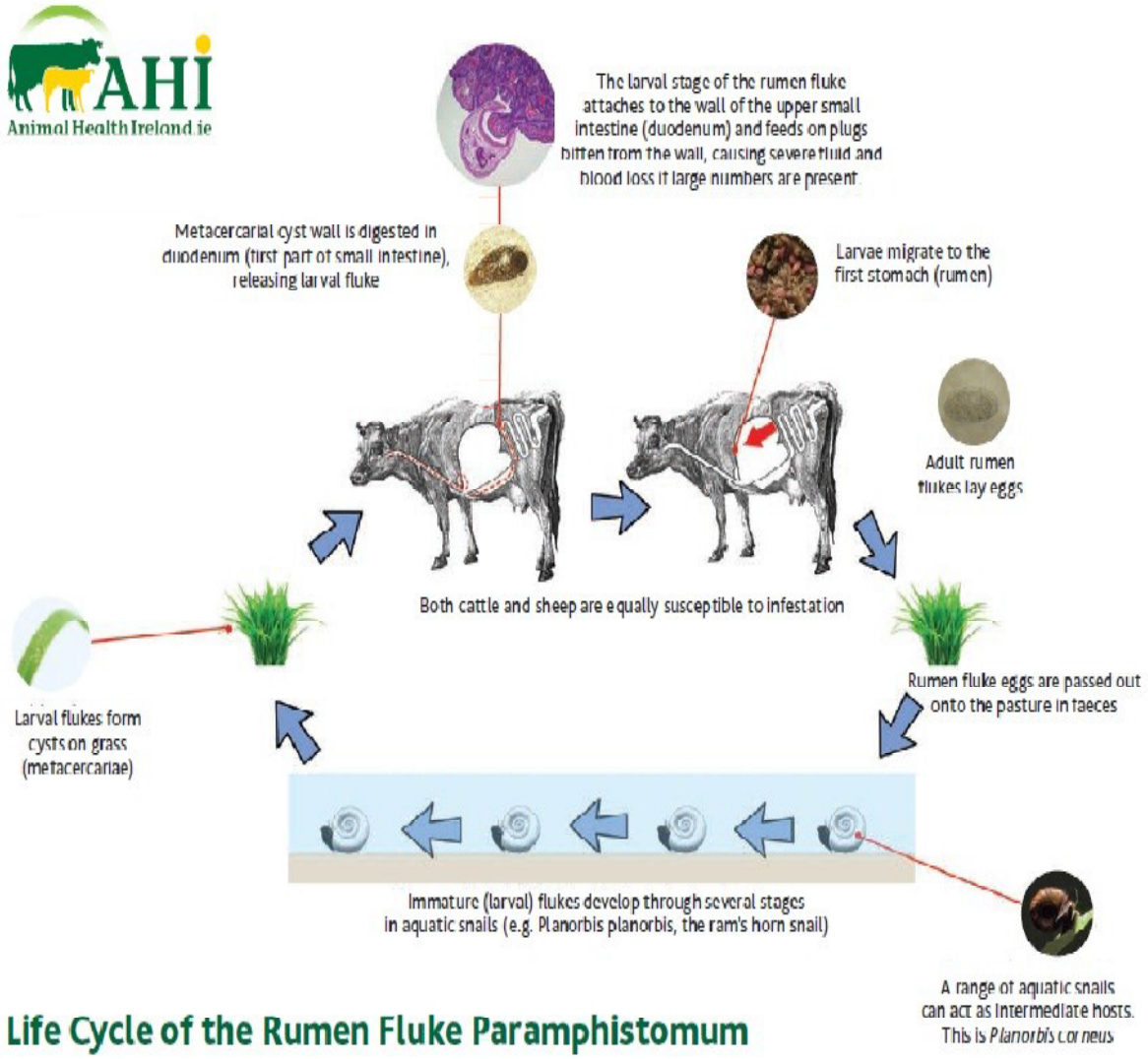


Figure 2: life cycle of *paramphistomum* parasite (parasitepedia 2013)

3.1. Classification:

According to Zeder (1790) *paramphistoma* was classified as follows:

Kingdom: *Animalia*

Phylum: *Platyhelminthes*

Class: *trematoda*

Subclass: *digenea*

Order: *Echinostomida*

Family: *paramphistomatidae*

Genus: *paramphistomum*

Cotylophoron

Calicophoron

Explanatum

Gigantocotyle

Ugandocycle

Type species: *P.cervi*

P.cotylophorum

P.microbothrium

P.gotoi

P.grande

P.hiberniae

P.ichikawai

P.epiclitum

3.2 Etiology:

Amphistomiasis in farm and wild mammals is due to infection of *paramphistomes*, such as the species of *Paramphistomum*, *Calicophoron*, *Cotylophoron*, *Pseudophisthodiscus*,et

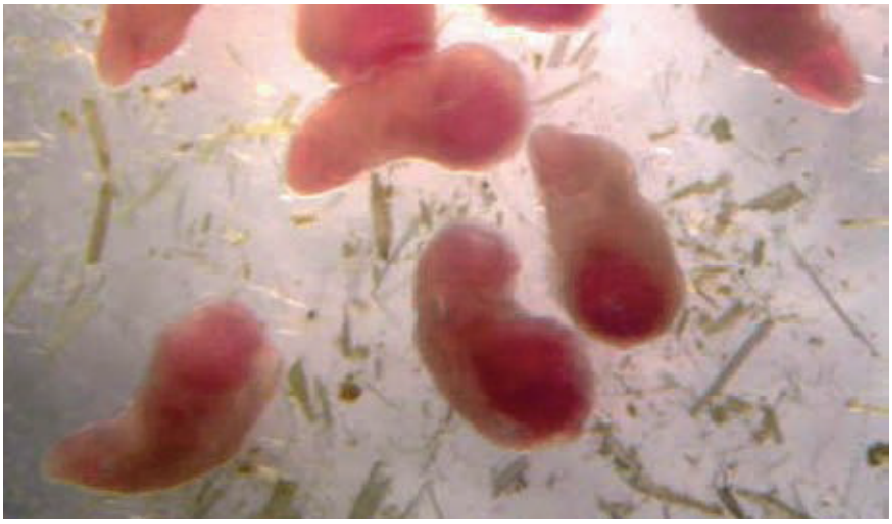


Figure3: Mature *Paramphistomum .spp* (Sanabria *et al* 2008)

These are essentially rumen flukes, of which *Paramphistomum.cervi* is the most notorious in terms of prevalence and pathogenicity. Infection occurs through ingestion of contaminated vegetables and raw meat, in which the viable infective metacercaria are reported from snails, which are the intermediate hosts (Chai et al.,2009) The immature flukes are responsible for destroying the mucosal walls of the alimentary tract on their way to growing into adults. It is by this tissue obliteration and appearances of clinical symptoms are manifested. The adult flukes, on the other hand, are quite harmless, as they merely prepare for reproduction (Brown .D.S. 2005)

3.3 Description of *paramphistoma* warm:

The generic name (Greek: para meaning "similar" [to *Amphistoma*], amphi meaning "on both sides", and stoma for "mouth") is given due to the presence of an anterior oral sucker and a posterior larger ventral sucker in adult worms (Boray 1959). The body is minute, measuring less than a centimeter. The body is covered with a highly folded tegument, which in turn is provided with sensory papillae.

Paramphistomum are all hermaphrodite, having both male and female reproductive systems in the posterior region of the body (Olsen, O.W. 1974).

4. Pathogenesis & Clinical signs:

Adult flukes are known to be quite harmless, as they do not attack on the host tissue. It is the mature flukes which are most damaging as they get attached to the intestinal wall, literally and actively sloughing off of the tissue. This necrosis is indicated by hemorrhage in feces, which in turn is a sign of severe enteritis. Under such condition the animals become anorexic and lethargic. It is often accompanied by pronounced diarrhea, dehydration, edema, polydipsia, anemia, listlessness and weight loss. In fact there are intermittent reports of mortality as high as 80% among cattle. Sometimes chronic form is also seen with severe emaciation, anemia, and rough coat mucosal edema in the sub maxillary space. The terminally sick animals lie prostrate on the ground, completely emaciated until they die. Most livestock have only light stomach fluke infections. They show no signs of disease due to presence of adult flukes or small numbers of immature fluke. The major clinical signs of stomach flukes are enteritis (inflammation of the small intestine) and strong diarrhea (watery scour) with blood traces, and as a consequences dehydration, dullness weight loss, etc. Anemia and bottle jaw also develop. Heavy infections with the immature fluke may cause decreased appetite, listlessness and weight loss. Fluid, foul-smelling diarrhea, dehydration and may terminate in death of the animal. Moderate infections with the immature fluke may cause reduced weight gains or milk production, or ill-thrift. Immature fluke live in the small intestine of ruminants where they attach themselves to the intestinal mucosa with powerful suckers. In large numbers, they destroy part of the mucosa and

cause acute inflammation of the intestine. Death may occur in severe infections (NSW 2007).

5. Diagnosis:

Provisional diagnosis is usually made on history and clinical signs of the disease (anorexia and projectile diarrhea) and the presence of immature *paramphistomes* in the fluid feces or at post mortem examination. Fecal examination for eggs at this stage is usually unrewarding as the disease is in the prepatent phase. Immature flukes are conical, pink in color and 1-5mm long. The fecal sedimentation technique, commonly used for *Fasciola* diagnosis is the most suitable for identify the eggs in feces. The eggs are oval and opercula, resembling that of *F. hepatica*; however, they are slightly larger and clear (transparent) rather than yellow in color. The addition of a contrast stain such as methylene blue may help to differentiate these two species of eggs. The adult flukes are pear-shaped and red in color, approximately 1cm long with a sucker at the tip of the cone and another sucker ventrally at the posterior-end (Waal, 2011).

ELISA is being practiced as the most effective diagnostic technique for detection of anti-parasitic antibodies (Shabih et al., 2006). Indirect plate enzyme-linked immune sorbent assay was standardized and evaluated for its effectiveness in immunodiagnostic of paramphistomosis in experimental and clinical cases in sheep, goat, cattle and buffaloes by using somatic whole adult antigen of *Paramphistomum epiclitum* and *Gastrothylax crumenifer*. Plate enzyme linked immune sorbent assay (ELISA) was standardized using 2 μ g/ml of antigen concentration with 1:200 and 1:1,000 of sera and conjugate dilution. Indirect Plate ELISA was able to demonstrate the antibody titer at different weeks post infection in experimental cattle Indirect ELISA described by (Estuningsih

et al., 2004) with some modification, The antigen was diluted in coating buffer at optimal concentration, optimal concentration was determined after preparation of different antigen concentration and different dilutions of sera and conjugate (chequer-board titrations).Immune response at weekly interval varied in all the group of experimental cattle (Kaur *et al*., 2009)

6. Postmortem:

At post mortem there is marked hemorrhagic enteritis with large numbers of the immature worm parasites on the mucosa or contents of the duodenum and upper ileum, subcutaneous edema, gelatinous fatty degeneration. Extensive catarrhal or hemorrhagic duodenitis or jejunitis with destruction of associated glands and lymph nodes are the main histopathological features. Immature flukes may be found embedded in the duodenal mucosa. There is marked fall in total plasma proteins due to increased leakage of plasma albumin (Kusiluka et al., 1996).

7. Treatment:

Most drugs licensed for the treatment of *fasciolosis* are not effective against *paramphistomosis*. Only a few drugs have been shown to have an efficacy against either the immature and/or mature flukes namely; niclosamide, oxyclozanide, rafoxinide and resorantel. However, since paramphistomiasis is seldom a significant economic problem in many temperate climates, these drugs are often not available and/or licensed for the treatment of *paramphistomes*. In Ireland, oxyclozanide is the only practical option for the treatment (Wall, 2011)

8. Prevention and control:

Where rumen flukes are endemic, preventive measures are a must to reduce the snail populations, the infection of pastures with infective stages, or the access to livestock to highly infested pastures. Vector snails are aquatic and live in water (e.g. streams, lakes, pools, swamps, marshes, irrigation channels, ditches, ponds, watering holes, water lopping, etc.) and are enormously prolific. Whatever measures help keeping the pastures dry are encouraged, either to reduce the snail population, or to shorten the survival time of encysted metacercariae, e.g.:

· Ensuring an adequate drainage

· Building watering points on solid ground, without puddles

· Make unavoidable ditches or channels less attractive to the snails by:

Making the borders steeper and/or cover them with concrete to eliminate the surrounding vegetation, drying them completely out periodically, etc.

· eliminate small water points that support the snails, e.g. hardened footprints (of shoes or car tires).

If permanent humid environments cannot be eliminated, they have to be fenced to prevent livestock from grazing there. Livestock infected with rumen flukes can develop a certain level of natural immunity that will make them more resistant to massive attacks by young flukes. Keeping livestock healthy and well fed diminishes the harm caused by rumen flukes and favors the development of the previously mentioned natural immunity. There are so far no vaccines against rumen flukes. Biological control of rumen flukes (i.e. using their natural enemies) is so not feasible. (F.A.O. 2009).

9. Epidemiology:

Flooding, caused by heavy rains, results in the dispersal of snails from permanent water masses, such as lakes and ponds. Paramphistomum eggs, deposited in these areas by grazing animals, hatch and infect the snails.

Outbreaks of disease generally occur in the dry months of the year when the receding water uncovers herbage contaminated with encysted metacercariae in these areas. In the UK, it has been suggested that dispersal of snails by flooding events and changes in farm-management practices may be responsible for the apparent emergence of the parasite (Foster et al., 2008). Previous infection and the age of the host animal afford some protection against reinfection. Acute disease is usually seen in young animal less than two years of age, older (adult) animals often continue to harbor for snails Sheep appear susceptible throughout their lives and multiple infections only result in partial immunity to reinfection.(Waal, 2011).

10. Geographic distribution:

Paramphistomum parasite is considered as worldwide in prevalence. It is most commonly found in tropical and subtropical regions, including Australia, Asia, Africa, Easter Europe, and Russia. The most debilitating cases are reported in Europe from Bulgaria, Italy, France, and Poland and also in Asia from Thailand, India, and China. The parasitic infection was first described from Punjab, India (Boray., 1959).

11. Previous Studies:

A study was conducted to investigate the prevalence and potential risk factors of *paramphistomiasis* in Sudan in White Nile State in Rabak slaughterhouse of 156 of cattle during 2014, the disease was diagnosed by

conventional method, fecal sedimentation test and by use (ELISA). The results showed high sero-prevalence rate by ELISA test (53.2%), compared to the much lower prevalence by fecal sedimentation test (29.5%). The risk factors associated with *paramphistomiasis* were: breed, grazing type, body condition, water source, snail presence, water bodies, knowledge of owner about disease, manure disposal and other disease with fecal sedimentation test and sex, water source, vegetation, manure disposal, schistosomaiasis and other disease.(Motasim.,2014)

A cross-sectional study was conducted to determine the prevalence in Abyei area – Sudan) in which the prevalence of paramphistomiasis in cattle was 11.25% (Gad alkareem *et. al*, 2012).

A study was carried out to determine the prevalence and intensity of *paramphistomiasis* in native sheep from Mazanderan province, in the north of Iran in association with sex, age, breed and season. During the 4 seasons of 2008, at meat inspection the rumen and reticulum of 132 native cattle and 104 mixed breed were examined by naked eye for *paramphistomiasis*. The result obtained showed overall prevalence rate as 33.9% *paramphistomes* per animal, 40.9% in sheep, and 25% in mixed breeds, respectively. A few *paramphistomes* were collected from the reticulum of a native sheep. There was no significant relation between the intensity of the infection and breed (P=0.094). Age and the infection (P=0.016) were significant. The older group (5 ≤) harbored more trematodes than ≤2 and 3–4-year-old, and p-values: P=0.026 and P=0.032 were significant, respectively. (Eslami *et.al*.,2011). A study was conducted to investigate the prevalence of parasitic diseases in different abattoirs in selective area of Bangladesh. Animals were examined for post-mortem changes in different abattoirs of those districts. The study started from February, 2008 to August, 2008. The total number of

animals examined was 3510, among them 1460 cattle, 620 buffaloes, 970 goats and 460 sheep. Age, sex and breed of the examined animals were recorded. The overall prevalence of hydatidosis was highest (26.01%) followed by fascioliasis (20.74%), and *amphistomiasis* (19.62%). The prevalence of the above mentioned diseases was higher in older animals. The prevalence of hydatidosis, fascioliasis and *amphistomiasis* was higher in male in cattle and goats, but the prevalence of those diseases was distinctly higher in female animals' buffaloes and sheep. The proportional prevalence of different disease conditions in cattle was much higher in Hariana breed than those of local and crossbred cattle. (Raza *et al.,* 2009)

A retrospective study was carried out over a 10- to 12-years period to analyze the changes in prevalence of natural fasciolosis and *paramphistomosis* among cattle and snails in central France, and to determine the causes which had induced these changes. The prevalence of natural fasciolosis in cattle increased from 1990 to 1993 (13.6% to 25.2%) and diminished afterwards up to 1999 (at 12.6%). Those of natural *paramphistomosis* showed a progressive increase between 1990 and 1999 (from 5.2 to 44.7%). The prevalences of natural infections and the numbers of free rediae counted in the snails (*Lymnaea truncatula*) infected with *F.hepatica* did not show any significant variations over time. By contrast, the prevalences of natural *paramphistomosis* in snails significantly increased from 1996 to 2000 and remained after wards in the same range of values (3.7‑5.3%), while the number of free rediae significantly increased up to 2006 (from a mean of 6.5 to 13.8 rediae per infected snail, respectively) (Magea. *et al*., 2002).

A cross sectional study was carried out with the aim of determining the prevalence and intensity (worm burden) of *Paramphistomum* in ruminants slaughtered from October, 2010 to April, 2011 at Hashim

Nur▪s Ethiopian Livestock and Meat Export industrialized abattoir in Debre Zeit, Ethiopia. One thousand one hundred fifty two ruminants comprising cattle, sheep and goats (n=384 each) were subjected to routine post mortem examination for the presence of *Paramphistomum*. The overall prevalence of *Paramphistomum* infection in the study proved to be 28.6 % (329/1152) of which 154 (40.1 %) were in cattle, 111 (28.9 %) in sheep and 64 (16.7 %) in goats. the highest prevalence of *paramphistomosis* was registered in highland goats, 30.2% (116/384) compared to those originated from lowland, 15.4 % (59/384). In the current study the prevalence proved to be higher in adult goats than young goats with prevalence of 30.5 % (117/384) in adult and 15.1% (58/384) in young goats. Infection was found to be highest in poor body condition (76.3 %), followed by medium (23.9 %) and good (6.9 %) body conditioned animals. A statistically significant difference ($p<0.05$) of *Paramphistomosis* prevalence was observed on the basis of species, body condition, different age groups and agro climatic zones (origins) of shoats. (Melaku, *et al*.2012).

A cross-sectional study was conducted to determine the prevalence and risk factors associated with small ruminant helminthiasis in north Gondar zone, northwest Ethiopia from November-January, 2008. A total of 558 small ruminants (458 sheep and 100 goats) were examined using standard parasitological procedures. The study revealed that the overall prevalence of helminthiasis was 47.67%. The species level prevalence of helminthiasis was 46.07% and 55% in sheep and goats, respectively. Sex and age of the animals were found to have association with prevalence but significant differences were not found. Therefore during control and treatment of small ruminant helminthiasis agroecology, species, age and sex of the animals should be considered as potential risk factors for the occurrence of the disease in the study areas (Dagnachew . 2008)

An epidemiological survey of *paramphistomosis* in ruminants in different districts of Punjab was conducted during the year 2005-2006 under DST, New Delhi sponsored project. A total of 1941 fecal samples (351 cattle, 791 buffaloes, 435 sheep and 364 goats) were collected from different village(s)/area(s) of the district of Punjab (Faridkot, Jalandhar, Ludhiana, Mansa, Muktsar, Nawanshahar and Sangroor). The samples were tested for *paramphistome* eggs by sedimentation method. Out of the total, 44 fecal samples (25 buffaloes, 7 cattle, 9 sheep and 3 goats) were found positive for *paramphistome* eggs with an incidence rate of 2.27%. The highest incidence was found in buffaloes (3.16%) followed by sheep (2.07%), cattle (1.99%) and goats (0.82%) in different district of Punjab. District-wise incidence rate was observed to be highest in Faridkot (7.4%) followed by Muktsar (2.37%), Mansa (2.3%), Sangroor (2.2%), Jalandhar (1.3%), Nawanshahar (1.3%), and Ludhiana (0.71%). Overall, seasonal epidemiology revealed highest incidence during monsoon with the incidence rate of 3.07% followed by 1.23% in winter, 0.6% in post-monsoon and 0.56% in summer (Shabih *et al*., P.2006).

Another study was designed to compare information on parasitic diseases occurrence in nomadic cattle herds in Abyei area. Fecal and blood samples collected from animals over one year. Fecal samples, blood smear, ticks and biting flies were collected over year. The results showed that the faecal samples from cattle examined by floatation and sedimentation methods showed that: *Paramphistomum* sp. constituted 11.25%, *Fasciola gigantica* 5.00%, *Schistosoma bovis*, 1.50%, *Oesophagustomum sp*. 2.50%, *Moniezia sp*. 0.63% and *Eimeria sp*. 4.38%. The occurrence of internal parasites was found higher during the wet season (Gad Alkareem *et al*., 2012).

A cross-sectional study was conducted to determine the prevalence and risk factors associated with The IgG antibody response to

Calicophoron daubneyi (Digenea: Paramphistomidae) excretory/secretory antigens was evaluated in naturally infected cattle from Lugo (Galicia, NW Spain) by using an ELISA procedure. Five hundred .twenty four belong to the age group was surveyed G-1 (0‑2 years old), G-2 (3‑5 years old) and G-3 (>6 years old). The ELISA procedure showed that 61.2% of the cattle in the study had been exposed to the trematode, but only 10.1% passed eggs in the feces. (Shabih et al. P.D.2006).

To investigate the prevalence of amphistome parasites in Black Bengal goats slaughtered at different slaughterhouses of Mymensingh district, a total of 144 gastro-intestinal tracts were examined during the period of July 1998 to June 1999, Bangladesh Agricultural University, Mymensingh. Out of 144 Black Bengal goats, 105 (72.92%) were infected with a single or multiple species of *amphistomes*. Age had a significant ($p<0.01$) influence on the prevalence of *amphistomes* in goat. A higher prevalence (89.58%) was observed in old animals followed by young ones (78.57%), whereas a lower prevalence (45.0%) was recorded in growing animals. However, the prevalence increased with the increase of age. Female animals (75.0%) were found more (1.44 times) susceptible to *amphistomes* infection than males (67.5%). The prevalence of *amphistomes* was very high all the year round and the rate of infection was 83.64%, 69.23% and 64.0% during monsoon, winter and summer season respectively. It was concluded that Black Bengal goats are susceptible to *amphistome* infection irrespective of age, sex and season of the year. (Uddin *et al* 2006).

A Survey of prevalence and fluke burden of *Paramphistomum sp.* was conducted among the major ruminants slaughtered in Sokoto in Nigeria Central Abattoir between May and October, 2007. One hundred (100) of each was examined for the presence of *Paramphistomum* species (stomach flukes). Flukes were counted to determine the average fluke

burden and prevalence. Out of the 300 animals, a total of 100 animals (33.3%) were infected with average fluke burden of 4794. Among which, 56 were cattle, with fluke burden of 2517(52.5%}, (32%) were sheep with fluke burden of 1907 (39.8%) and 12 goats with fluke burden of 370. and Out of 100 cattle, 20 (20%) males and 36 (36%) females were infected with flukes. Also, out of the 100 sheep, 4 (4%) were males and 28 (28%} were females and in goats, 4 (4%) were males and 8 (8%) were females. On the basis of age the results showed that 4 cattle(7.1%) out of the 56 infected animals were 1-2 years, 40 (71.4%) were 3-4 years old and 12 (21.4%) were >4 years . Of the 32 infected sheep, 6 (18.7%) were 1-2 years, 18(56.2%) were 3-4 years and 8(25%) were >4yrs. Similarly, 2(16%) out of the 12 goats infected were 1-2 years, 8 (66.6%) were 3-4 years old and 2 (16%) were >4 years. The result obtained showed that *Paramphistomiasis* is prevalent in the cattle in the area, with female cattle having higher prevalence.(Abunza *et al* 2008)

In another study investigating the role of snail in lifecycle of *paramphitoma* single-miracidium infections of *Lymnaea truncatula* with *Paramphistomum daubneyi* or with *Fasciola hepatica* were carried out under laboratory conditions to count free rediae, their germinal embryos, and to determine the cercarial productivity of each redial generation. In snails infected by P. daubneyi, the cercariae were produced by the first (8.7 cercariae per redia) and second (8.9 per redia) generations. At day 63 post-exposure, they corresponded, respectively, to 53.9% and 46.1% of cercariae produced by all rediae. In snails infected by F. hepatica, the majority of cercariae were produced by the R2a group (18.2 cercariae per redia) and corresponded to 66.0% of cercariae produced all rediae.The cercariae produced by the other redial groups were more limited in number: 17.5 per redia in the R1b group (28.7%) and 2.0 per redia in the R2b/R3a group (5.3%). Cercarial productivity of *P. daubneyi* until day 63

post-exposure was more limited in number than that of F. hepatica: a total of 145 cercariae per snail versus 427 per snail.(Abrous,D. *et al*., 2000).

In another study of italian Isolates of the rumen fluke *Calicophoron daubneyi* (Digenea: Paramphistomidae) from various hosts in three locations in southern Italy were characterized genetically. The second internal transcribed spacer (ITS-2) of ribosomal DNA (rDNA) plus flanking 5.8S and 28S sequence (ITS-2+) was amplified from individual rumen flukes by PCR. PCR-linked restriction fragment length polymorphism (PCR-RFLP) analysis was performed using four different restriction endonucleases, and PCR products were sequenced. The PCR analyses from all the C. daubneyi specimens produced identical fragments, and the PCR-RFLP analyses did not show, among of the four restriction endonucleases, the differences between the C. daubneyi specimens. The sequence analyses of the ITS-2+ fromeach of the C. daubneyi specimens showed that all of them 428 bp, and composed of the entire ITS-2 sequence (282 bp) plus the two partial flanking conserved sequences, 5.8S (99 bp) and 28S (47 bp). No intra-specific variation was observed in the nucleotide composition of the ITS-2+ (homology = 100%). There was, however, an observable interspecific variation between the ITS-2+ of *C. daubneyi* and the ITS-2+ of both *Calicophoron calicophorum* (homology = 97.2 %) and *Calicophoron microbothrioides* (homology = 97.4 %), both previously deposited in the GenBankTM. The findings of the obtained study showed that, ITS-2 can serve as an effective genetic marker for the molecular identification of *paramphistomes*, and as a useful tool for developing molecular epidemiological techniques for the study of C.daubneyi transmission patterns and prevalence in definitive and intermediate hosts (Rinaldi *et al*., 2005).

Ageographic information system (GIS) was constructed using remote sensing (RS) and landscape feature data together with *Calicophoron daubneyi* positive survey records from 197 georeferenced ovine farms with animals pasturing in a 3971 km2 area of the southern Italian Apennines. The objective was to study the spatial distribution of this rumen fluke, identify environmental features that influence its distribution, and develop a preliminary risk assessment model. The GIS for the study area was constructed utilizing the following environmental variables: normalized difference vegetation index (NDVI), land cover, elevation, slope, aspect, and total length of rivers. These variables were then calculated for "buffer zones "consisting of the areas included in a circle of 3 km diameter centered on 197 farms. The environmental data obtained from GIS and RS and from data taken by the veterinarians on the field (stocking rate and presence of streams, springs and brooks on pasture) were analyzed by univariate (Spearman and ANOVA) and multivariate (discriminant) statistical analyses using the farm coprological status (positive/negative) as the dependent variable. Sheep on 32 of the 197 (16.2%) farms were positive for *C.daubneyi*, with an average intensity of 52 epg (Cringoli *et al*., 2004).

In an attempt to establish an ideal method for mass production of *Calicophoron microbothrium* metacercariae, a study was carried out to compare the shedding capacities of *Bulinus tropicus* naturally and experimentally infected with *C. microbothrium*. A total of 906 F1 B. tropicus between 4 and 5 weeks old were each experimentally infected with two *C. microbothrium* miracidia and monitored for 12 weeks. The infected snails were fed on dried lettuce and fish flakes and were kept in 1 l plastic aquaria housed in a snail room where temperature, light and humidity were controlled. Seventy-four percent of the experimentally infected snails died during the prepatent period and of the remaining, only

13.2 % developed patent infection, while 12.5 % were refractory. Snail growth rate was poor and the average shedding rate was 20 cercariae per snail per day (Mavenyengwai *et al*., 2006).

Rumen of 100 slaughtered animals viz. sheep (n=14), goats (n=42), cattle (n=34) and buffalo (n=10) were examined to determine the prevalence of adult *Paramphistomum cervi* during January 2007 in Tehsil Jatoi, District Muzaffar Garh, Pakistan. Overall prevalence was found to be 22% (22/100) and species wise prevalence was 28.57% (4/14) in sheep, 23.80% (10/42) in goats, 17.64% (6/34) in cattle and 20% (2/10) in buffaloes, the difference between the species was not significant. (Raza et al 2009). To investigate the Epidemiology of *Paramphistomum* infection in cattle, fecal samples from 360 cattle were collected from individual areas of the Sirajgonj district from March 2009 to April 2010. One hundred and ninety one animals (53.1%) were infected with single or multiple species of *Paramphistomum*. Age of animals significantly ($P<0.05$) influenced the prevalence of *Paramphistomiasis*. Older animals suffered (60.3%) more than growing (44.4%) and young (54.0%) ones. Older animals were 1.94 times more susceptible than growing animals. Furthermore, females were more (59.5%; 1.79 times) susceptible to *Paramphistamum* spp. than males (45%). Breed has also significant ($p<0.05$) effect. The prevalence of *Paramphistomiasis* was higher ($p<0.05$) in crossbred (61.8%) animals than that of local (49.2%) cattle. (Paul *et.al* 2011).

The analysis of infection by *Paramphistomidae* trematodes was conducted in two agricultural regions with different knowledge on this parasitosis. Faecal and blood samples were collected from 374 cattle in Salto (NW Uruguay) where there is a lack of information about *paramphistomosis*. A total of 429 cattle from Galicia (NW Spain), he percentage of cattle passing Paramphistomidae - eggs by feces was 7%

(95% confidence Interval 5, 10). A significantly higher prevalence of *paramphistomosis* in the Hereford Angus cattle (OR = 3.5) was recorded (Sanchis *et al*., 2013).

A cross sectional study was carried out from October 2010 to March 2011 at Andassa Livestock Research Center, North-West Ethiopia. The objective was to determine the prevalence of cattle fluke's infection. Fecal samples were collected from a total of 384 cattle, cross breed (n= 39) and Fogera breed (n=345) of all age groups and sex. Sedimentation technique was employed for the recovery of fluke eggs from freshly collected fecal sample. The results indicated that the overall prevalence of bovine fluke's infection was 60.42%. In this study, the highest prevalence was recorded from *Paramphistomosis* (45.83%) followed by Fasciolosis (23.96%), and Schistosomosis (9.89%). (Yeneneh *et al*., 2012).

Epidemiological studies were undertaken at slaughter houses, livestock farms, and veterinary hospitals and on house hold buffaloes under different management and climatic conditions in four different districts of the Punjab province. Infection rate was 7.83%, 12.33%, 7.17% and 4.25% respectively in the cattle at the slaughter house, livestock farm, and veterinary hospital and at household cattle. Overall the highest prevalence in terms of season, 26% and 14.50%, was recorded during autumn at livestock farms and slaughtered cattle followed by 9.75% veterinary hospitals during summer and the lowest (2.5%) in household cattle was recorded during winter. (Khan *et al* 2008).

A Survey of prevalence and fluke burden of *Paramphistomum sp.* was conducted among the major ruminants slaughtered in Sokoto Central Abattoir between May and October, 2007. One hundred (100) of goats, sheep and cattle each were examined for the presence of *Paramphistomum* species (stomach flukes). Flukes were counted to determine the average fluke burden and prevalence. Out of the 300

animals, a total of 100 (33.3%) were infected with an average fluke burden of 4794. Out of these, 56 (56%) were cattle, with fluke burden of 2517(52.5%}, 32 (32%) were sheep with fluke burden of 1907 (39.8%) and 12 (12%} with fluke burden of 370 (6.7%) were goats. Out of the 100 cattle, 20 (20%) males and 36 (36%) females were infected with flukes. Also, out of the 100 sheep, 4 (4%) were males and 28 (28%} were females and in goats, 4 (4%) were males while 8 (8%) were females. On the basis of age the result showed that 4(7.1%) out of the 56 infected animals were those of 1-2 yrs, 40 (71.4%} were 3-4 yrs old and 12 (21.4%) were animals >4 yrs in respect of cattle. Of the 32 infected sheep, 6 (18.7%) were 1-2 yrs, 18(56.2%) were 3-4 yrs and 8(25%) were >4yrs. Similarly, 2(16%) out of the 12 goats infected were 1-2 yrs, 8 (66.6%) were 3-4 yrs old and 2 (16%) were >4 yrs (Abunza *et al*., 2008).

Another study explored various basic aspects of the epidemiology of *paramphistomosis* in Galicia, the main cattle producing region in Spain. A total of 589 cows from different farms located across the region were selected at random in the slaughterhouse for examination of the rumens and reticula for the presence of *Paramphistomidae* flukes. *Paramphistomes* were found in 111 of 589 necropsied cows (18.8%; 95% CI: 15.7%-21.9%), with higher prevalences of infection in beef cows than in dairy cows (29.2% vs 13.9%). Although the number of flukes per animal was generally low (median= 266 flukes), some cows harbored large parasite burdens (up to 11895 flukes), which may have harmful effects on their health or productivity (Gonz . 2012).

Chapter Two

Materials and Methods

2.1 Study Area:

Study was conducted on (Kadaru) slaughter house, (Khartoum State) Khartoum State has strategic location, and it lies in the central of the Sudan, lies between longitudes 31.5 to 34 ° E and latitudes 15 to 16 ° N. Population of the state was estimated 5,274,321 in 2008 census of about 639,598 urban and 5,274,321 person. The potential of Khartoum area for grazing is fewest, and mostly dependant on the location of vegetation and water located on the edges of the state areas. The estimated livestock cattle in Khartoum state is 38.3% of the Sudan's livestock (M.A.R.F.P., 2014).

Kadaru slaughter house located in Khartoum Bahri locality (importance location that most of cattle exported or slaughtered in this slaughter house (M.A.R.F.P, 2014). In this study most of animals that slaughtered came from Koko market, Almowaeleh market and from small farms.

The Climatic condition in Khartoum state like most of Sudan has a very dry climate. The climate stays hot throughout the year.

2.2 The study design:

The study design was a cross sectional study which provided information on occurrence of paramphistomiasis (Martin *et.al*, 1987). A Cross-sectional study was conducted at Kadaru abattoir on two regular days a week (Monday – Thursday) in winter (December to February 2015) the animals in these days were selected by random sampling method.

2.3 Sample Size:

The expected prevalence of cattle paramphistomiasis for calculation of sample size was taken from the study done in Sudan (where the prevalence of paramphistomiasis estimated 29.5% in White Nile State

Sample size was calculated according to the formula by Martin *et. , al*.(1988).

$$n = \frac{4 P Q}{L2}$$

Where:

N= Required Sample Size P= Expected prevalence

Q = 1- P L= Allowable error = 0.0025

$$\frac{4\times29\times71\times10.000}{100\times100\times25} = 330 \text{ animal}$$

2.4. Individual risk factors:

Potential individual risk factors and their categories were as follow:

Sex (male, female), age (adult, young), breed (local and cross), body condition (good, poor).

2.5 Management risk factors:

Management risk factors included: Grazing type (indoor, outdoor), source of animals (Niala, East state), water source (tap, river), knowledge of owner about disease (yes, no), fasciola (positive, negative), schistiosoma (positive, negative), and other disease (positive, negative), treatment of the disease (yes, no).

2.6 Animals and sample collection:

A Survey of paramphistomiasis in slaughter house was done according to the questionnaire. Rectal fecal samples were collected in the abattoir of Kadaru (Khartoum State, Sudan). The majority of the slaughtered cattle were beef cattle and minorities were culled dairy cows. After collection, samples were transported to the laboratory. The feces were stored by adding formalin 10% (Adejoju *et al*.,2008).

2.7 Diagnostic technique:

Fecal Examination:

Fecal samples (approximately 10 gram) were collected directly from the rectum of the animal in a clean plastic container after labeling with specific identification number; each sample was transported to Veterinary Research Laboratory. Fecal samples were examined by sedimentation technique for the presence of fluke eggs using the method described by (Adejoju *et al*., 2008). The technique was performed on 10 g of feces to which. 200 ml water was added and mixed. The mixture was filtered 3 times through a specific sieve. The filtrate was allowed to stand for 10 min after which the sediment was collected in a test tube and centrifuged at 1000 rpm for 3 min. After centrifugation, the supernatant was decanted and a drop of the sediment was tested microscopically. Trematode eggs were identified on the basis of morphology （Soulsby, 1982）.

Figure 4: Eggs of *Paramphistomum cervi* (P) and *Fasciola hepatica* (F) (wall.2012)

2.8 Statistical Analysis:

Results of the study were analyzed using Statistical Package of Social Science (SPSS). First, Descriptive statistical analysis was displayed in frequency distribution and cross tabulation table. Univariate analysis using the chi-square for qualitative data. P-value of number was considered as significant association and the risk factor was then selected to enter the multivariate analysis. Multivariate analysis: Forward or backward stepwise logistic regression was used to analyze the data and to investigate association between a potential risk factor and the prevalence of *paramphistomiasis*. A p-value of 0.05 indicated significant association between *paramphistomiasis* and the risk factor.

Chapter Three

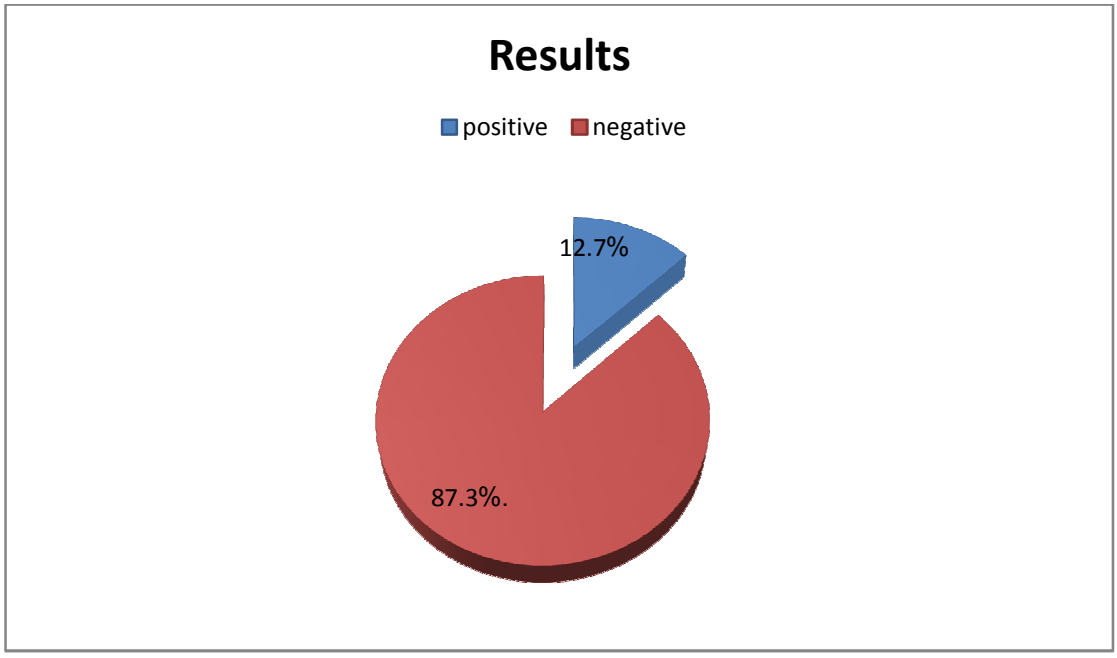
Results

3.1 Results:

Of the total 330 cattle inspected, 42 (12.7%) animals were positive, and the rest were negative for *paramphistomiasis* (table 3.1). the overall prevalence of cattle *paramphistomiasis* examined by fecal sedimentation test in *Kadaru* was 12.7%.

Table 3.1: Distribution of paramphistomiasis infection among 330 cattle examined by fecal sedimentation test in Kadaru slaughterhouse:

Valid	Frequency	Percent	Percent Valid %	Percent Cumulative%
+ve	42	12.7	12.7	12.7
-ve	288	87.3	87.3	100
Total	330	100	100	



3.2 Age of animals:

Three hundred Thirty three cattle of various ages were examined in this study. The result showed that the age distribution of cattle, 82 (24.8%) were young (less or equal 3 years) and 248 (75.2%) of cattle were more than 3 years (old), (table 3.2). Among young animals 9 animals were found infected. The rate of infection within young animals was 11%. Among adults 33 animals was found infected. The rate of infection within adults was 13.3% (table 3.3). The Chi- square test showed no significant association between *paramphistomum* infection and age of animal (p-value = 0.583), (table 3.4).

3.3 Sex of animals:

The results of this study as shown in table 3.2/3.3 the distribution of 330 cattle examined for *paramphistoiasis* according to sex. Total number of male examined was 309 (93.6%) animals, while the total number of female examined was 21 (6.4%) (Table 3.2) among males, 35 animals were found infected. Rate of infection within males was (11.3) %. While among females, 7 animals were found infected. The rate of infection within females was (33.3) % (table 3.3). The Chi- square test showed significant association between *paramphistomum* infection and sex of animal (p-value = .003), (table 3.4).

3.4 Breed:

The distribution of paramphistomiasis infection in Kadaru slaughter house according to breeds is shown in table 3.2 /3.3. Total number of local breed was 294 (89.1%) animal. Among these 294 animals, 36 animals were found infected. The rate of infection was 12.2%. Total number of cross breed examined was 39 (10.1%). Among these, there were 6 infections. The rate of infection was 16.7% (table 3.1.3). The Chi-square test showed no significant association between the infection and breed (p-value=0.452), (table3.4).

3.5 Body condition:

The body condition of animals and the presence of infection were investigated. 323 (97.9%) of cattle were found to be in good condition, while 7 (2.1%) of cattle were found to be in poor condition (table 3.1.2). Among good condition animals 39 were found infected. The rate of infection within good animals was 12.1%. While three animals were found infected among poor condition animals. The rate of infection within poor animals was 42.9 % (table 3.3). The Chi- square test showed a significant association between the infection and body condition (p-value = 0. 016), (table 3.4).

3.6 Source of animal:

Of the total 330 cattle inspected, 237 animals were from *Niala* where were 30 infected, 93 were from the West stats. Among these 93 animals, 12 were found infected .The rate of infection in *Niala* was (12.7 %). And the rate of infection in west Stats was (28.2 %), (table3.3). The Chi-square results showed no significant association between the infection and source of animal (p-value = 0.952), (table 3.4).

3.7 Water source:

The distribution of paramphistoiasis infection in *Kadaru* slaughter house according to water source was shown in tables (3.3) and (3.4). Total number of animals (330) drinking from water taps. However 42 animals were infected. The rate of infection was 12.7%. (Table3.3). The Chi- square test did not show significant differences because water source was a constant, (table 3.4).

3.8 Knowledge about the disease:

The distribution of *paramphistoiasis* infection in Kadaru slaughter house according to knowledge about the disease was shown in tables (3.3) and (3.4). The total numbers of animals´ owners who had well knowledge about the disease were (330). However 42 animals were

infected. The rate of infection was 12.7%. (Table3.3). The Chi- square test did not show any results because knowledge about the disease was a constant, (table 3.4).

3.9 Fasciola:

The distribution of *paramphistoiasis* infection in Kadaru slaughter house according to fasciola infection was shown in tables (3.3) and (3.4). Total numbers of animals with negative fasciolaisis were 327 animals, while the total numbers of animals with positive fasciolasis were 3 animals (table3.3). The rate of infection with negative fasciolasis was (99.1%). The rate of infection with positive fascioliasis was (0.9 %). The Chi- square test showed significant association between the *paramphistoiasis* infection and fascioliasis (p-value = 0.005), (table 3.4).

3.10 Schistosoma:

The distribution of *paramphistoiasis* infection in *Kadaru* slaughter house according to *schistosoma* infection was shown in tables (3.3) and (3.4). Total number of animals that not infected (-ve *schistosomiasis*) were (330). However 42 animals were infected of *paramphistoiasis*. The rate of infection was 12.7%. (Table 3.3). The Chi- square test did not show any results because schistosoma was a constant, (table 3.4).

3.11 Other diseases:

The distribution of paramphistoiasis infection in Kadaru slaughter house according to other diseases was shown in tables (3.3) and (3.4). Total number of animals with negatives other diseases were 328 (99.4 %) animals. while the total number of animals with positive to other diseases were 2 (0.6 %) animals (table3.3). Among these negative to other diseases 42 animals found infected with *paramphistoiasis*. The rate of infection in negative to other diseases was (12.7 %). Among these positive to other diseases no animals were found infected with

paramphistomiasis .The rate of infection with positive to other diseases was (0%). The Chi- square test showed no significant association between the infection of *paramphistomiasis* and other diseases (p-value = 0.588), (table 3.4).

3.12 Treatment:

The distribution of *paramphistomiasis* infection in *Kadaru* slaughter house according to treatment was shown in tables (3.3) and (3.4). Total numbers of animals´ owners (330) were found to use treatment. However 42 animals were infected with *paramphistomiasis*. The rate of *paramphistomiasis* was 12.7%. (Table3.3). The Chi- square test did not show any results because treatment was a constant, (table 3.4).

3.13 Grazing:

The distribution of *paramphistomiasis* infection in *Kadaru* slaughter house according to grazing was shown in tables (3.3) and (3.4).Total numbers of animals (330) grazed indoor. However 42 animals were infected with *paramphistomiasis*. The rate of infection was 12.7%. (Table3.3). The Chi- square test did not show any results because grazing was a constant, (Table 3.4).

The study showed significant association between *paramphistomiasis* and three potential risk factors; sex, body condition and fasciola infection in multivariate analysis (Table 3.5). The odds ratio (Exp - B) to the risk factor sex was 3.627 if male put as a reference, which means the infection of *paramphistomiasis* in females equal 3.627 times in males with confident interval 95% for exponent –B (1.259 -10.446) (Table 3.5). The odds ratio (Exp - B) to the risk factor body condition was 1.057 if a bad condition put as a reference, which means the infection of *paramphistomiasis* in bad condition equal 1.057 times in a good condition with confident interval 95% for exponent –B (0.090 -12.450)

(Table 3.5). The odds ratio (Exp - B) to the risk factor fasciola infection was 0.084 if the negative infection of fasciolasis put as a reference, which means the infection of *paramphistomiasis* in positive cases of fasciolasis equal 0.084 times in negative infection of fasciolasis with confident interval 95% for exponent –B (0.003 - 2.664) (Table 3.5).

Table 3.2: Summary of frequency distribution of 330 cattle from Kadaru slaughterhouse examined for paramphistomiasis by fecal sedimentation test according to potential risk factors:

Risk Factors	Frequency	Relative Frequency %	Cumulative Frequency %
Sex			
Female	**21**	**6.4**	**6.4**
Male	**309**	**93.6**	**100**
Total	**330**	**100**	
Age			
Young (≤3)	**82**	**24.8**	**24.8**
Adult(>3)	**248**	**75.2**	**100**
Total	**330**	**100**	
Breed			
Local	**294**	**89.1**	**89.9**
Cross	**39**	**10.1**	**100**
Total	**330**	**100**	
Body condition			
Good	**323**	**97.9**	**97.9**
bad	**7**	**2.1**	**100**
Total	**330**	**100**	

Continue to Table 3.2			
Source of Animal Niala west stats Total	**237** **93** **330**	**71.8** **28.2** **100**	**71.8** **100**
water source Tap River Total	**330** **0** **330**	**100**	**100**
knowledge of ownner about disease Yes No Total	**330** **0** **330**	**100**	**100**
fasciola disease -ve +ve Total	**327** **3** **330**	**99.1** **0.9** **100**	**99.1** **100**
Schistisoma disease -ve +ve Total	**330** **0** **330**	**100**	**100**
Other disease -ve +ve Total	**328** **2** **330**	**99.4** **0.6** **100**	**99.4** **100**

Continue to Table 3.2			
Treatment of disease Yes No Total	**330** **0** **330**	**100**	**100**
Grazing Type Indoor Outdoor Total	**330** **0** **330**	**100**	**100**

Table 3.3: Summary of cross tabulation for the rate of paramphistomiasis in each category of the potential risk factors in 330 cattle from Kadaru slaughterhouse examined by fecal sedimentstion test:

Risk factors	No. inspected	No. affected (%)
Age		
Young	82	9 (11)
Old	248	33 (13.3)
Sex		
Female	21	7 (33.3)
Male	309	35 (11.3)
Breed		
Local	294	36 (12.2)
Cross	36	6 (16.7)
Body condition		
Good	323	39 (12.1)
Bad	7	3 (42.9)

Continue to Table 3.3		
Source of animal		
Niala	237	30 (12.7)
West stats	93	12 (12.9)
water source		
Tap	330	42 (12.7)
River	0	0
Knowledge of owner about disease		
Yes	330	42 (12.7)
No	0	0
Fasciola		
+ve	3	2 (66.7)
-ve	327	40 (12.2)
Schistisoma		
+ve	0	0
-ve	330	42 (12.7)
Other disease		
+ve	2	0
-ve	328	42 (12.7)
Treatment		
Yes	330	42 (12.7)
No	0	0
Grazing type		
Indoor	330	42 (12.7)
Outdoor	0	0

Table 3.4: Summary univariate analysis for the association between paramphistomiasis and potential risk factors in 330 cattle examined at Kadaru slaughterhouse by fecal sedimentation test using the Chi_square test:

Risk factors	No. inspected	No. affected (%)	d.f	X2 value	p- value
Age			1	0.301	0.583
Young	82	9 (11)			
Old	248	33 (13.3)			
Sex			1	8.573	.003*
Female	21	7 (33.3)			
Male	309	35 (11.3)			
Breed			1	0.565	0.452
Local	294	36 (12.2)			
Cross	36	6 (16.7)			
Body condition			1	5.845	.016
Good	323	39 (12.1)			
Bad	7	3 (42.9)			
Source of animal			1	0.004	0.952
Niala	237	30 (12.7)			
West stats	93	12 (12.9)			
water source			1	Risk factor is a constant	-
Tap	330	42 (12.7)			
River	0	0			
Knowledge of owner about disease			1	Risk factor is a constant	-
Yes	330	42 (12.7)			
No	0	0			
Fasciola			1	7.930	0.005
+ve	3	2 (66.7)			
-ve	327	40 (12.2)			

Continue to Table 3.4					
Schistisoma +ve -ve	 0 330	 0 42 (12.7)	1	Risk factor is a constant	-
Other disease +ve -ve	 2 328	 0 42 (12.8)	1	0.293	.588
Treatment Yes No	 330 0	 42 (12.7) 0	1	Risk factor is a constant	-
Grazing type Indoor Outdoor	 330 0	 42 (12.7) 0	1	Risk factor is a constant	-

* Mean significant value

Table 3.5: multivariate analysis for the association between paramphistomiasis and potential risk factors in 330 cattle examined at Kadaru slaughterhouse by fecal sedimentation test:

Risk Factors	**No. inspected**	**No. affected%**	Exp(B)	P-value	95% CI for EXP (B)	
					Lower	**Upper**
Sex Female Male	 21 309	 7 (33.3) 35 (11.3)	 3.627 Ref	0.003	1.259	10.446
Body condition Good Bad	 323 7	 39 (12.1) 3 (42.9)	1.057 Ref	0.016	0.090	12.450
Fasciola +ve -ve	3 327	2 (66.7) 40 (12.2)	0.084 Ref	0.005	0.003	2.664

* Mean significant value

Chapter Four

Discussion

The present study has increased knowledge on the epidemiology of *paramphistomiasis* in cattle in kadaru Slaughterhouse in Khartoum State of the Sudan, there was investigated by using questionnaires and fecal sedimentation test. Fecal sedimentation showed that the prevalence rate of *paramphistomiasis* was12.7% in the study area compared with other States of the Sudan which is less than other States. The study included investigations on the potential risk factors contributing to the occurrence and spread of *paramphistomiasis* among cattle populations, the overall prevalence rate of egg of *paramphistomiasis* in cattle fecal samples collected from Kadaru slaughterhouse in Khartoum state were found to be 12.7% (42/288) by fecal sedimentation test. The results obtained from fecal sedimentation in the present study was higher than the prevalence reported by a numbers of authors (GadAlkareem *et al* (2012) in Sudan who reported prevalence of 11.25% (18/160) in cattle, Sanchis *et al* (2013) in Spain who reported prevalence of 7% (803/56), Krishna *et al.,*(2013) in India, who reported prevalence of 1.99% (351/7) , Dı ´ az, *et al*.(2006) in Spain, who reported prevalence of 10.1% (524/53), Khan *et al* (2008) in India, who reported prevalence of 7.83% (188/2400), and Shabih *et .al .P.*(2006) in India who reported prevalence of 3.4% (651/22). However the prevalence reported in the present study was lower than the prevalences reported in Bangladesh of 53.1% (360/191) Paul *et al* (2011) and in Ethiopia of 45.8 % (176/384) Yenenehet al (2012). And in Ethiopia of 44.23% (46/104) by Fromsa *et al* (2011). This could be explained by the differences in the tested sample size (n), practicing of traditional communal grazing and geographical regions, elaborated by the differences in the tested sample size (n), animal production systems and

geographical regions. Knowledge of risk factors associated with paraphistomiasis in cattle is an important pre-requisite for the design and implementation of effective control strategies and for management programs that can lead to the control and eradication of the disease. Knowledge of these risk factors and their association and contributions to the occurrence and spreading of *paraphistomiasis* among cattle populations also is a good aid for clinical diagnosis and for determining the epidemiology and patterns of the disease. In the current study, univariate analysis using Chi -square, with a confidence interval of 95% at a p-value of ≤0.25 was used to identify potential risk factors associated with fecal sedimentation test positivity for *paraphistomiasis* infection in cattle. Significant risk factors associated with fecal sedimentation test positive in the univariate analysis were found to be Age (x^2= 0.301, p = 0.583), Sex (x^2 = 8.573, p = 0.003), Breed (x^2=0.565 , p =0.452), Body condition (x^2 =5.845, p = 0.016), Source of animal (x^2 = 0.004, p = 0.952), fasciola (x^2= 7.930, p = 0.005), Other diseases (x^2= 0.293, p =0.588).

The positive association of sex with fecal sedimentation test paramphistomiasis-positivity in cattle is in agreement with the findings of Krishna *et al* (2013), while the positive association of body condition, grazing type, water source, knowledge of owner about the disease, and other disease, with fecal sedimentation test paramphistomiasis-positivity in cattle are investigated for the first time. This positive association of sex as risk factor could be explained by the fact that males are known for its tolerance to parasitic diseases. The multivariate analysis, using logistic regression, with a confidence interval of 95% and a p- value of ≤0.05 was used to assess the association between identified significant risk factors in the univariate analysis in combination towards a positive fecal

sedimentation test status for paramphistomiasis in cattle. However, some potential risk factors which were regarded to be important with $p \leq 0.25$ in the univariate analysis were also entered into the multivariate analysis. This analysis showed an association between being fecal sedimentation test positive status for *paramphistomiasis* infection in cattle and sex(Exp (B) =3.627), body condition (Exp (B) = 1.057), fasciola (Exp (B)= 0,084), positive association of body condition with fecal sedimentation test *paramphistomiasis*-positivity in cattle is in agreement with the findings by Fromsa *et al* (2011), whilst the positive association of body condition as risk factor could be explained by the fact that the fluke causes high protein loses in ruminant and also the emaciated animal has lower resistance to fluke than cattle with a good body condition.

Conclusion:

It could be concluded that bovine *paramphistomiasis* according to fecal sedimentation test diagnosis is prevailing in Kadaru slaughterhouse of Khartoum State with prevalence value 12.7 % (42/330).

Based on the results of this cross sectional study, the potential risk factors associated with *paramphistomiasis* in cattle in kadaru slaughterhouse of Khartoum State were reported as: Sex, body condition and fasciola.

Recommendations:

1- More studies on potential risk factors that enhance the spread and transmission of *paramphistomiasis* in cattle in the Sudan.

2- Integrated control and eradication program should be launched as recommended by OIE.

3- The scheme of initiation of a regional network for surveillance, control and eradication of this important disease in the surrounding Africa countries.

4- Screening test in large markets like *Almowalih* market and *kuku* market should be done by local veterinary authorities to prevent and control the disease.

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Appendix

Questionnaire:

Investigation of paramphistomiasis in Khartoum State.

Conducted by: The preventive medicine Department of Sudan university of science & technology, Faculty of veterinary medicine.

Locality_____ date _____

Animal/s Owner_____

Herd.Code_____

Address _____ __

1-The individual risk factors:

1-Age :(years)

Young ( )

Adult ( )

2-sex:

Male ( )

Female ( )

3-Breed:

Local ( )

Cross ( )

4-Body condition:

Good ( )

Poor ( )

5- Previous history of the disease:

Yes ( )

No ( )

Comment:

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2- Management risk factors:

6- Source of animal:

Kordofan ( )

White Nile ( )

7-Grazing type:

Indoor ( ) Outdoor ( )

8-water source:

Tap ( )

Canal ( )

River ( )

9- snail presence:

Yes ( ) No ( )

10- water bodies:

Yes ( ) No ( )

11- Vegetation:

Yes ( ) No ( )

12- Knowledge of owner about disease:

Yes ( ) No ( )

13- manure disposal:

Yes ( ) No ( )

14- Fasciola:

Positive ( ) Negative ( )

15- Schitiosoma:

Positive ( ) Negative ( )

16- Other disease:

Positive ( ) Negative ( )

17-Tretment of disease:

Yes ( ) No ( )

Comment:……

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