

بسم الله الرحمن الرحيم

**Sudan University of Science and  
Technology  
College of Graduate Studies**

**Prevalence and Risk factors of Cattle  
Paramphistomiasis in Omdurman locality,  
Khartoum State, Sudan**

نسبة الإنتشار وعوامل الخطر لمرض البارامفيستوميسيس فى الأبقار  
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***To my father***

***To my mother***

***To my daughters and my  
sons***

***To my brother and sisters***

***To my husband***

***To my colleagues and  
friends***

***To all who  
have helped me .***

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### **List of abbreviations**

ELISA	Enzyme linked immune sorbent assay
mg	Ml gram
Kg	Kilo garm
OR	Odds ratio
°E	East
°N	North
gm	Gram
°C	Centigrade
min	minute
rpm	Round per minute
ml	Millimeter
spp	Species
No.	number
d.f	degree of freedom



X <sup>2</sup> value	chi -square value
Exp(B)	odds ratio
P value	Probability value
C.I	confidence interval
Ref	Reference
OIE	World organization for animal health

### **Abstract**

Across-sectional study was carried out on 333 of cattle in Ganawa slaughterhouse in Omdurman locality, Khartoum state, Sudan, during winter (December 2014 - January 2015). The objectives of this 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 21.9% when tested by fecal sedimentation test. The prevalence of the infection according to the age was 36.4% in animals equal and less than three years and 21.4% more than three years. The prevalence according to the sex was 21.9% for male. The prevalence according to the breed of the animals was 21.9% for local. The prevalence according to the body condition was 20.8% for good condition and 83.3% for poor condition. The prevalence according to the source of the animals was 22.2% from East Darfur and 21.5% from South Darfur, the prevalence according to the fasciola infection was 12.3%. and the prevalence according to the Schistosoma infection was 0.0% and the prevalence according to the treatment of the disease was 24.9% for no used of treatment and 7.1% for used of treatment.

Univariate analysis using the Chi-square, with confidence intervals of 95% at a  $p$ -value  $\leq 0.25$  was used to identify potential risk factors associated with fecal sedimentation test- positivity for paramphistomiasis in cattle. Significant positive risk factors associated with fecal sedimentation test in the univariate analysis, there were found to be age ( $x^2 = 1.386$ ,  $p$ -value = 0.239), body condition ( $x^2 = 13.463$ ,  $p$ -value = 0.000), fasciola ( $x^2 = 7.623$ ,  $p$ -value = 0.006), Treatment of the disease ( $x^2 = 8.591$ ,  $p$ -value = 0.003), There were also to be significant risk factors associated with fecal sedimentation test 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 in Cattle, the analysis showed association between the paramphistomiasis in Cattle and in age (Exp (B) = 1.003), body condition (Exp (B) = 0.056), fasciola (Exp (B) = 0.310) and treatment of the disease (Exp (B) = 3.629).

It could be conclude that the potential risk factors (age, body condition, fasciola and treatment of the disease) were showed highly significant association with paramphistomiasis.

### ملخص الدراسة

أجريت دراسة مقطعية لعدد 333 رأس من الأبقار في ولاية الخرطوم, محلية أمدرمان مسلخ قناوى, خلال فصل الشتاء اعتباراً من ديسمبر 2014 وحتى يناير 2015, كان الهدف من هذه الدراسة هو تقدير معدل انتشار مرض دودة الكرش في الأبقار والتحقق من عوامل الخطر المرتبطة بانتشار مرض دودة الكرش.

كان معدل انتشار المرض في كل الحيوانات التي تم فحصها باختبار ترسيب البراز هو 21.9%. كان معدل انتشار العدوى وفقاً لسن الماشية 36.4% في الحيوانات الأقل أو تساوى ثلاث سنوات و 21.4% للحيوانات الأكثر من ثلاث سنوات. وكان معدل الانتشار وفقاً لجنس الحيوان 21.9% فى الذكور(كل الحيوانات التي تم فحصها كانت من الذكور وذلك لقانون منع ذبح الإناث), ووفقاً لسلالة الحيوان كان معدل الانتشار 21.9% للسلالة المحلية(كل الحيوانات التي تم فحصها كانت محلية), أما بالنسبة لحالة الجسم فكان معدل الانتشار 20.8% للحالات الجيدة 83.3% للحالات الغير-جيدة , وكان معدل الانتشار وفقاً للمناطق التي جاءت منها الحيوانات 22.2% من شرق دارفور و 21.5% من جنوب دارفور , ووفقاً للإصابة بالفاشيولا كان معدل الانتشار 12.3% و كان معدل الانتشار وفقاً للحيوانات التي لم يستخدم معها الأدوية 24.9% و . تم التحقق من عوامل الخطر الإيجابية المرتبطة بالمرض باستخدام مربع كاي للتحويل في التحليل وحيد المتغير  $p\text{-value} = 25.0 \geq$  حيث كانت عوامل الخطر المرتبطة بانتشار المرض هي:

العمر, ( $x^2 = 1.386, p\text{-value} = 0.239$ ) وحالة الجسم, ( $x^2 = 13.463, p\text{-}$ ) و ( $\text{value} = 0.000$ ) الإصابة بالفاشيولا, ( $x^2 = 7.623, p\text{-value} = 0.006$ ) و أدوية المرض ( $x^2 = 8.591, p\text{-value} = 0.003$ )

باستخدام التحليل بالانحدار اللوجستي  $p\text{-value} \leq 0.05$  لمعرفة درجة الارتباط بين-انتشار المرض وعوامل الخطر , أظهرت النتائج وجود ارتباط إيجابى بين-مرض دودة الكرش

و حالة (Exp (B) = 0.056) وعمر الحيوان (Exp (B) = 1.003) للمرض ،  
(Exp (B) = 3.629) وفى حالة الإصابة بالفاشيولا (Exp (B) = 0.310) الجسم

أظهرت هذه الدراسة وجود ارتباط وثيق بين معدل انتشار دودة الكرش فى الأبقار والعمر  
وحالة الجسم و الإصابة بالفاشيولا واستخدام الأدوية.

## Introduction

*Paramphistomum* (rumen fluke disease) is one of the common parasites in the rumen and reticulum of sheep, goats, cattle and water buffaloes. *Paramphistomum* in duodenum and ileum are plug feeders and cause hemorrhage which leads to bleeding and diarrhea. Bleeding for prolonged period may cause anemia, which further weakens the host. 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. 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). Acute paramphistomiasis usually occurs in young cattle less than two years of age and is characterized by listlessness, anorexia and profuse diarrhea develops two to 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 and other parasitic diseases) (Waal, 2011). 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). 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.*,2011).

*Paramphistomum* parasite has an indirect life cycle with fresh water snails as the intermediate hosts, e.g. the genus *Bulinus*, *Planorbis*, *Stagnicola* (Figure1).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 2weeks 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 (Figure2).

### **Scientific justification:**

Paramphistomiasis is an important neglected disease. It is considered as a public health problem in Africa, especially in rural communities. In Sudan, paramphistomiasis could be one of the major infectious diseases because most abattoirs in rural areas of the Sudan are not well qualified, and sheep, cattle and goats are still slaughtered traditionally. Determination of the prevalence of the disease in is very important in order to estimate the problem which may help in the control of the disease.

### **Objectives:**

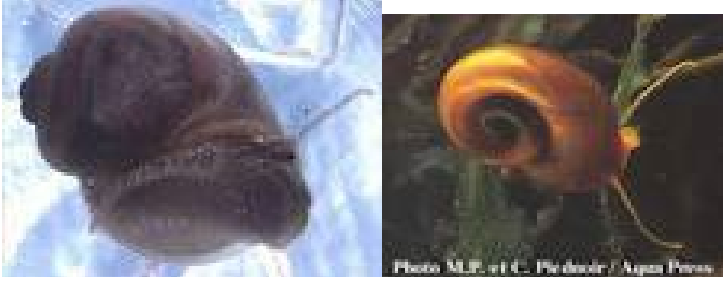
The objectives of this study were:

- To estimate the prevalence of bovine paramphistomiasis in cattle slaughtered in Ganawa slaughterhouse.

- To investigate the potential risk factors associated with the disease.







1-*Galba truncatula*

2- *Bulinustruncatus*

3-

*Planorbisplanorbi*

**Figure1:** Intermediate host of *paramphistomum spp.*  
(Source : [http://www. Pharma-unilim.fr](http://www.Pharma-unilim.fr)(le 15 Octobre 2012.)



## **1.1 Classification:**

According to Fiscoeder F (1904). *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*

## **1.2 Etiology:**

*Paramphistomum spp* are essentially rumen flukes, of which *Paramphistomum cervi* is the most notorious in terms of prevalence and pathogenicity. Infection occurs when ruminants ingest contaminated vegetables and raw meat contain infective metacercaria (chai, *et al.*,2009) The immature flukes are responsible for destroying the mucosa of the gut wall to grow into adults causing tissue obliteration and appearance of clinical symptoms. The adult flukes are quite harmless, as they merely prepare for reproduction (brown, *et al.*,2005 ).

### **1.3 Description:**

*Paramphistomum* means similar on both sides of 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 measure less than a centimeter and covered with a highly folded tegument, which in turn is provided with sensory papillae. *Paramphistomum* are monoecious self-fertilizes having both male and female reproductive systems in the posterior region of the body (Olsen,1974) (Figure3) .

### **1.4 Clinical signs:**

Small numbers of *paramphistomes*, adult or immature fluke, don't show any signs. Heavy infection with the immature flukes may cause decreased appetite, listlessness and weight loss fluid, foul-smelling diarrhea and dehydration

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 (NSW DPI 2007).

### **1.5 Diagnosis:**

Under most situations, infection is hard to recognize because the symptoms are mild or even absent. There is not yet a standard diagnostic test. Therefore, manual diagnosis is done at many levels. Diagnosis basically relies on a combination of [postmortem](#) analyses, clinical signs displayed by the animals. In heavy infection, symptoms are easily observed in sheep and cattle as they become severely [anorexic](#) or inefficiently digest food, and become unthrifty. Copious fetid diarrhea is an obvious indication, as the soiling of hind legs and tails with fluid feces are readily noticeable (Kumar, 1998). Even though it is not always the case, immature flukes can be identified from the fluid excrement. On rare occasions, eggs can be identified from stools of suspected animals (Olsen, 1974). In developing countries diagnosis and [prognosis](#) is often hindered by multiple infection with other trematodes, such as *Fasciola hepatica*

and [schistosomes](#), because these flukes are given primary importance due to their pervasive nature (Phiri, *et al.*, 2006).

Enzyme linked immune sorbent assay (ELISA) is being practiced as the most effective diagnostic technique for detection of anti-parasitic antibodies (Shabih, *et al.*, 2006). Indirect plateenzyme-linked immune sorbent assay was standardized and evaluated for its effectiveness in immuno diagnostic of paramphistomiasis in experimental and clinical cases in sheep, goat, cattle and buffaloes by using somatic whole adult antigen of *Paramphistomum epiclitum* (Kaur, *et al.*, 2009)

### **1.6 Postmortem:**

At post mortem, marked hemorrhagic enteritis with large numbers of the immature worm parasites could be observed on the mucosa or contents of the duodenum and upper ileum as well as subcutaneous edema and 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 (Kusiluka, *et al.*, 1996)

### **1.7 Treatment and prevention:**

There is no especial drug for treatment and control. However, some drugs were found to be effective for

treatment of the disease. Include resorantel, [oxyclozanide](#), clorsulon, [ivermectin](#), [niclosamide](#), bithional and [levamisole](#) (Bowman and Georgi 2008). An in vitro demonstration shows that [plumbagin](#) exhibits high efficacy on adult flukes (Saowakon, *et al.*,2013). Drugs effective against the immature flukes are recommended for drenching. For this reason oxyclozanide is advocated as the drug of choice. It effectively [kills](#) the flukes within a few hours and it is effective against the flukes [resistant](#) to other drugs. The commercially prescribed dosage is 5 mg/kg body weight or 18.7 mg/kg body weight in two divided dose within 72 hours. (Hugh-Jones, *et al.*,2008).

### **1.8 Epidemiology:**

Floods, caused by heavy rains, result in the dispersal of snails from permanent water masses, such as lakes and ponds. *Paramphistome* 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 United Kingdom, 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 while 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).

### **1.9 Geographic distribution:**

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

### **1.10 Previous Studies:**

A cross section study was conducted to investigate the prevalence and potential risk factors of paramphistomiasis in Sudan in White Nile State in Rabak slaughterhouse during 2014, the disease was diagnosed by conventional methods, fecal sedimentation test, and 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, schistosomiasis and other disease with ELISA (Motasim, 2014).

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 native sheep and mixed breed were examined by naked eye for paramphistomiasis. The result obtained showed overall prevalence rate 33.9% *paramphistomes* per animal, 40.9% in sheep and 25% in mixed breeds, respectively (Eslami, *et al.*, 2011).

A retrospective study was carried out over a 10- to 12-years period in central France Paramphistomiasis showed a progressive increase between 1990 and 1999 (from 5.2 to 44.7%). The prevalence of natural paramphistomiasis in snails significantly increased from 1996 to 2000 and remained afterwards in the same range of values (3.7–5.3) (Mage, *et al.*, 2006).

A cross sectional study was carried out from October, 2010 to April, 2011 at Hashim Nur's Ethiopian Livestock and Meat Export industrialized abattoir in Debre Zeit, Ethiopia. Ruminants comprising cattle, sheep and goats 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 % of which 40.1 % were in cattle, 28.9 % in sheep and 16.7 % in goats. The highest prevalence of paramphistomiasis was registered in highland goats, 30.2% compared to those originated from lowland, 15.4 % . In the current study the prevalence proved to be higher in adult goats than young goats with prevalence of 30.5 % in adult and 15.1% 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 Paramphistomiasis 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 in north Gondar zone, north west Ethiopia from November-January, 2008. Small ruminants (sheep and 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 a groecology, 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, et al., 2011).

An epidemiological survey of paramphistomiasis in ruminants indifferent districts of Punjab was conducted during the years 2005-2006, New Delhi sponsored project. Fecal samples were collected from different villages of the district of Punjab (Faridkot, Jalandhar, Ludhiana, Mansa, Muktsar, Nawanshahar and Sangroor). The samples were tested for *paramphistome* eggs by sedimentation method. 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. 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 ( Shabih1, et al., 2006).

To investigate the prevalence of *Paramphistome* parasites in Black Bengal goats slaughtered at different slaughterhouses of Mymensingh district were examined during the period of July 1998 to June 1999 in the Department of Parasitology, Bangladesh Agricultural University, Mymensingh. Black Bengal goats were infected with a single or multiple species of *Paramphistomes*. Age had a significant ( $p < 0.01$ ) influence on the prevalence of *Paramphistomes* in goat. A higher prevalence 89.58% was

observed in old animals followed by young ones 78.57%, where as 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 *paramphistomes* infection than males 67.5%. The prevalence of *Paramphistomes* 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 *Paramphistomes* infection irrespective of age, sex and season of the year. (Uddin, *et al.*, 2006).

A Survey of prevalence and fluke burden of *Paramphistomum spp.* Was conducted among the major ruminants slaughtered in Sokoto in Nigeria Central Abattoir between May and October, 2007. Ruminants were examined for the presence of *Paramphistomum* species total of 100 animals 33.3% were infected with average fluke burden. Cattle with fluke burden of 52.5%, 32% were sheep, 39.8% goats with fluke burden. And Out of 100 cattle, 20% males and 36% females were infected with flukes. Also, out of the 100 sheep, 4% were males and 28% were females and in goats, 4% were males and 8% were females. On the basis of age the results showed that cattle 7.1% were infected animals age were 1-2 years, 71.4% were 3-4 years old and 21.4% were >4years . In infected sheep, 18.7% were 1-2

years, 56.2% were 3-4 years and 25% were >4years. Similarly, 16% out of the 12 goats infected were 1-2 years, 66.6% were 3-4 years old and 16% were >4years. The result obtained showed that Paramphistomiasis is prevalent in the cattle in the area, with female cattle having higher prevalence (ABunza, et al.,2008).

Rumen of slaughtered animals. Sheep, Goats, cattle and buffalo were examined for adult *Paramphistomum cervi* during January 2007 in Tehsil Jatoi, District Muzaffar Garh, Pakistan. Overall prevalence was found to be 22% and species wise prevalence was 28.57% in sheep, 23.80% in goats, 17.64% in cattle and 20% 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 were collected from individual areas of the Sirajgonj district from March 2009 to April 2010. Animals 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. Furthermore, females 59.5% were more susceptible to *Paramphistomum 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).

A cattle from Galicia, The percentage of cattle passing Paramphistomidae-eggs by feces was 7% (95% Confidence Interval 5, 10). A significantly higher prevalence of paramphistomiasis 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. Fecal samples were collected from cattle, cross breed and Fogera breed 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 flukes infection was 60.42%. In this study, the highest prevalence was recorded from Paramphistomiasis 45.83% followed by Fascioliasis 23.96%, and Schistosomiasis 9.89% (Yeneneh, *et al.*, 2012).

A fecal samples (cattle, buffaloes, sheep and goats) were collected randomly from different villages of the district of Punjab and adjoining areas in Jammu during the period July 2004 to June 2005. The samples were screened microscopically for *paramphistome* eggs by sedimentation method. Fecal samples (buffaloes, cattle, sheep and goats) were found positive for *paramphistome* eggs with an incidence rate of 6.35 percent. The highest incidence was found in buffaloes followed by sheep, goats and cattle. District-wise incidence rate was observed to be highest in

Gurdaspur followed by Amritsar, Kapurthala and Jammu (shabeh, *et al.*, 2006).

Epidemiological studies were undertaken at slaughterhouses, livestock farms, veterinary hospitals and on household 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, 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).



**Figure 3:** Mature *Paramphistomum spp*

## **Chapter Two**

### **Materials and Methods**

#### **2.1 Study Area:**

This study was conducted at Ganawa slaughterhouse, Khartoum State .the capital city of the Sudan, it lies between longitudes 31.5 to 34 °E and latitudes 15 to 16 °N population



of the state was estimated at 5,274,321 in 2008 census of about 639,598 urban and 5,274,321 metro, The potential of Khartoum area for grazing is low. Grazing, therefore, is mostly dependent on the farms and water sources located on outside the state. The estimated cattle number in Khartoum state is 38.3% of the Sudan's livestock (Sudanow magazine 2014).

Ganawa slaughterhouse was chosen as it is one of the main slaughterhouses in the state.

## **2.2 The study design:**

This study was a cross sectional study to provide snap shot information on occurrence of paramphistomiasis in cattle slaughtered in Ganawa slaughterhouse in Khartoum State in the Sudan. Samples were collected on three randomly selected days in week. The animals in these days were selected by systematic random sampling method(Martin, *et al.*,1988). Among each group of five animals one animal was examined.

## **2.3 Sample Size:**

The sample size of study the expected prevalence of paramphistomiasis was estimated based on a previous study carried out by Mutasim (2014), who has estimate the prevalence of the disease in White Nile state, Sudan, at 29.5% (Muatsim 2014).

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

$$N = \frac{4 P^{\wedge} Q^{\wedge}}{L2}$$

**N** = sample size

$P^{\wedge}$  = expected prevalence

$L2$  = desired absolute precision

$Q^{\wedge}$  = (1- $P^{\wedge}$ ). (Martin , *et al.* 1988.)

$$\frac{4*29.5*70.5*10.000}{100*100*25} = \underline{333 \text{ animals}}$$

#### **2.4. Individual risk factors:**

Potential individual risk factors and their categories were designed to be as follow:

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

#### **2.5 Management risk factors:**

Management risk factors include: grazing type(indoor, outdoor), source of animals (East darfuor, South darfuor), water source (tap, river), snail presence (yes, no), water bodies (yes, no), vegetation (yes, no), knowledge of owner about disease (yes, no), manure disposal (yes, no), fasciola (positive, negative), schitiosoma (positive, negative), other disease (positive, negative) and treatment of the disease (yes, no) .

## **2.6 Sample collection:**

### **Survey of paramphistomiasis in slaughterhouse:**

Approximately 10 gm of fecal samples were collected at Ganawa slaughterhouse. Samples were collected directly from the rectum of the animal in a clean plastic container after labeling with specific identification number, transported to the laboratory and stored at 4°C until the test was performed within 48 hours.

## **2.7. Diagnostic technique:**

### **2.7.1. Fecal Examination:**

Fecal samples were examined by sedimentation method for the presence of fluke's eggs using the method described by (Adejoju, *et al.*, 2008). Briefly, 10 gm of feces were mixed with 200 ml of tap water in a test tube. The mixture was filtered 3 times through specific tea strainer. The filtrate was allowed to stand for 10 min after which the sediment was collected in a test tube and centrifuged at 700 rpm for 3 min. After centrifugation, the supernatant was decanted and a drop of the sediment was tested microscopically using a cover slip used Oil immersion 40× lens (Figure 4). Sample should be examined directly or be frozen (-20) or used chloroform 5% or formalin 10%, if will be examined later. Trematode's eggs were identified on the basis of morphology (Soulsb, 1982) (Figure 5).

### **2.7.2. Postmortem examination:**

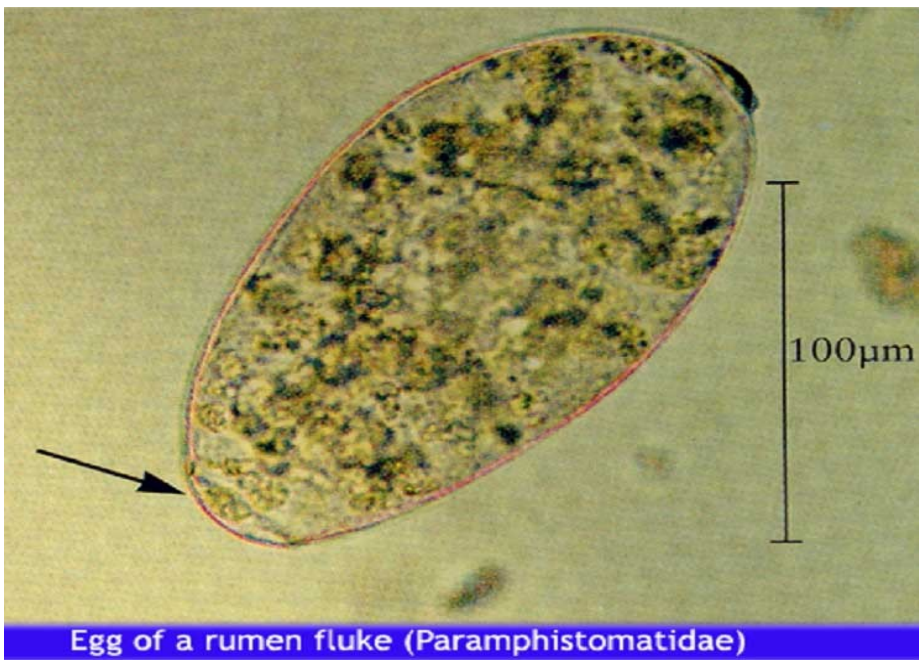
During meat inspection, the previously identified animals and their rumens were carefully supervised and examined, so as to avoid mixing up of the organs to be inspected and the fecal samples. The animals from which fecal samples were collected had been subjected to a rumen dissection after being slaughtered. The presence of mature flukes was done visually by the naked eye (Figure 6) .

### **2.8 Analysis of the results:**

Results of the study were analyzed using statistical package of social science (SPSS). First, descriptive statistical analysis was displayed infrequency distribution and cross tabulation tables. Univariate analysis was performed using chi-square for qualitative data. *P-value*  $\leq 0.25$  was considered as significant association and the risk factor 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*  $\leq 0.05$  indicated significant association between paramphistomiasis and the risk factor.



**Figure 4:** A drop of the sediment was tested microscopically (photographs personally).



**Figure 5:** Eggs of *Paramphistomum (P) cervi* (wall, 2012).



**Figure 6:** Mature of *paramphistomum spp* fixed in rumen (photographs personally).

## Chapter Three

### 3.1 Results:

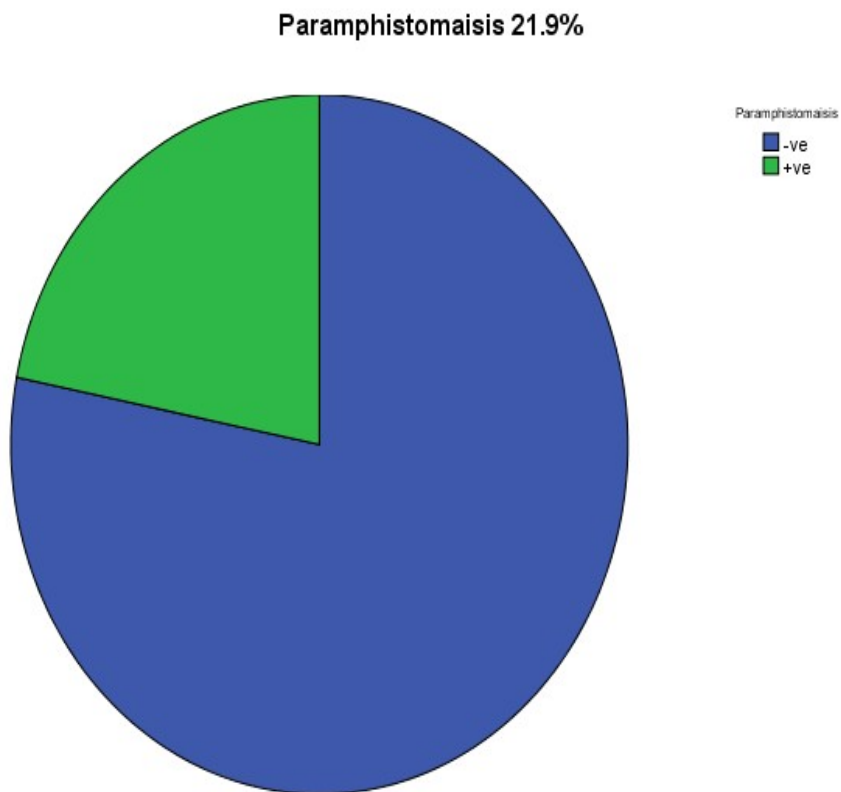
From the 333 examined cattle, 73 (21.9 %) were found to be positive for the mature fluke's during rumen inspection (Figure 7). This was confirmed by the presence of the fluke's eggs in the fecal samples collected from the same animals after being slaughtered. The eggs were observed under the light microscope as a part of the fecal sedimentation method (table 3.1).

**Table 3.1:** Distribution of paramphistomiasis among 333 cattle examined by fecal sedimentation method at Ganawa slaughterhouse, Omdurman locality, Khartoum state , Sudan.

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid				
-	260	78.1	78.1	78.1
ve	73	21.9	21.9	100.0
+				
ve				
Total	333	100.0	100.0	



## paramphstomiasis (21.9%)



+ve

-ve

**Figure 7:** Prevalence of paramphstomiasis (21.9%) at Ganawa slaughterhouse.

### **3.2 Age of animal:**

From the 333 samples were collected from different ages categorized as follows, young animals 11(3.3%), Adult animals 322(96.7%)(table 3.2). from the 11 young animals 4(36.4) were found to be positive for *paramphstome spp.* While from 322 adult animals 69(21.4) were found to be positive for *paramphstome spp.* Using both methods, fecal sedimentation method and rumen dissection (table 3.3). Chi2 test showed that young animals are more susceptible to paramphstomiasis (p- value= 0.239)( table 3.4).

### **3.3 Sex of animal:**

From the 333 samples were collected from animals. Total number of animals were male (table 3.2).

### **3.4 Breed:**

From the 333 samples were collected from animals. Total numbers of animals were local breed (table 3.2).

### **3.5 Body condition:**

From the 333 samples were collected from different body conditions categorized as follows, good condition animals 327(98.2%), poor condition animals 6(1.8%)(table 3.2). from the 327 good condition animals 68(20.8%.) were found to be positive for *paramphstome spp.* While from 6 poor condition animals 5(83.3%) were found to be positive for *paramphstome spp.* Using both methods, fecal sedimentation method and rumen dissection (table 3.3). Chi2 test showed that poor condition animals are more susceptible to paramphstomiasis (p- value= 0.000)( table 3.4).

### **3.6 Source of animal:**

From the 333 samples were collected from different regions categorized as follows, East Darfur 189(56.8%), south Darfur 144(43.2%)(table 3.2). from the 189 animals from East Darfur 42(22.2%.) were found to be positive for *paramphstome spp.* While from 144 animals from South Darfur 31(21.5%) were found to be positive for *paramphstome spp.* Using both methods, fecal sedimentation method and rumen dissection (table 3.3). Chi2 test showed that no significant association from paramphstomiasis (p- value= 0.879)( table 3.4).

### **3.7 Grazing:**

From the 333 samples were collected from type of grazing. Total number of animals were grazing indoor (table 3.2).

### **3.8 Water source:**

From the 333 samples were collected from water source. All animals were drinking from taps (table 3.2).

### **3.9 Presence of snails:**

From the 333 samples were collected from presence of snails. All animals with no presence of snails (table 3.2).

### **3.10 Presence of water bodies:**

From the 333 samples were collected from presence of water bodies. All animals with no presence of water bodies (table 3.2).

### **3.11Vegetation:**

From the 333 samples were collected from vegetation. All animals with no vegetation presence (table 3.2).

### **3.12 Knowledge of owner about disease:**

From the 333 samples were collected from different Knowledge of owner about disease categorized as follows, yes Knowledge of owner about disease 315(94.6%), no Knowledge of owner about disease 18(5.4%)(table 3.2). from the 315 yes Knowledge of owner about disease 71(22.5%) animals were found to be positive for *paramphstome spp.*

While from 18 no Knowledge of owner about disease 2(11.1%) animals were found to be positive for *paramphstome spp.* Using both methods, fecal sedimentation method and rumen dissection (table 3.3).

Chi2 test showed that no significant association to paramphstomiasis (p- value= 0.254)( table 3.4).

### **3.13 Manure disposal:**

From the 333 samples were collected from manure disposal. All animals with no manure disposal (table 3.2).

### **3.14 Fasciola:**

From the 333 samples were collected from different animals fasciolaiasis categorized as follows, positive fasciolaiasis animals 19(5.7%), negative fasciolaiasis animals 314(94.3%)(table 3.2). from the 19 positive fasciolaiasis animals 9(47.4%.) were found to be positive for *paramphstome spp.* While from 314 negative fasciolaiasis animals 64(20.3%) were found to be positive for *paramphstome spp.* Using both methods, fecal sedimentation method and rumen dissection (table 3.3).

Chi2 test showed that positive fasciolaiasis animals are more susceptible to paramphstomiasis (p- value= 0.006)( table 3.4).

### **3.15 Schistosoma:**

From the 333 samples were collected from different Schistosoma infction categorized as follows, positive Schistosomaiasis animal 1(0.3), negative Schistosomaiasis animals 332(99.7%)(table 3.2). from the 332 negative Schistosomaiasis animals 73(21.9%.) were found to be positive for *paramphstome spp.* (table 3.3).

Chi2 test showed that no significant association to paramphstomaisis (p- value= 0.596)( table 3.4).

### **3.16 Other diseases:**

From the 333 samples were collected from different animals with other diseases categorized as follows, positive other disease animals 2(0.6%), negative other disease animals 331(99.4%)(table 3.2). from the 2 positive other disease animals 1(50.0%.) was found to be positive for *paramphstome spp.* Wile from 331 negative other disease animals 72(21.8%) were found to be positive for *paramphstome spp.* Using both methods, fecal sedimentation method and rumen dissection (table 3.3).

Chi2 test showed that no significant association to paramphstomaisis (p- value= 0.336)( table 3.4).

### **3.17 Treatment of disease:**

From the 333 samples were collected from different use treatment of disease categorized as follows, yes use treatment of disease 56(16.8%) animals, no use treatment of disease 277(83.2%)(table 3.2). from the 56 yes use treatment of disease 4(7.1%.) animals were found to be positive for *paramphstome spp.* Wile from 277 no use treatment of disease 69(24.9%) animals were found to be positive for *paramphstome spp.* Using both methods, fecal sedimentation method and rumen dissection (table 3.3). Chi2 test showed that animals with no used treatment of disease are more susceptible to paramphstomiasis (p-value= 0.003)( table 3.4).

This study showed significant association between paramphstomiasis and four potential risk factors; age, body condition, fasciolaiasis and treatment of the disease in multivariate analysis (Table 3.5). The odds ratio (Exp - B) to the risk factor age was 1.003 in young with confident interval 95% for exponent -B (0.223 -4.515), (Table 3.5). The odds ratio (Exp - B) to the risk factor body condition was 0.056 in poor condition with confident interval 95% for exponent -B (0.006 -0.513), (Table 3.5). The odds ratio (Exp - B) to the risk factor fasciolaiasis was 0.310 in negative infection of fasciolaiasis with confident interval 95% for exponent -B (0.119 - 0.808), (Table 3.5). The odds ratio (Exp - B) to the risk factor treatment of disease was 3.629 in no

used of treatment with confident interval 95% for exponent - B (1.257 - 10.476) (Table 3.5).

**Table 3.2:** Summary of frequency distribution of 333 cattle examined for paramphistomiasis by fecal sedimentation method according to potential risk factors at Ganawa slaughterhouse, Omdurman locality, Khartoum state, Sudan.

<b>Risk factors</b>	<b>Frequen cy</b>	<b>Perce nt</b>	<b>Relative Percent%</b>	<b>Cumulati ve Percent%</b>
<b>Valid Age</b>	11	3.3	3.3	3.3
Young( $\leq 3$ )	322	96.7	96.7	100.0
Adlut				
( $> 3$ )				
<b>Sex</b>				
Mal	333	100.0	100.0	100.0
Fem	0			
<b>Breed</b>				
Local	333	100.0	100.0	100.0
Cross	0			
<b>Body condition</b>				
Poor	6	1.8	1.8	1.8
Good	327	98.2	98.2	100.0
<b>Suorce of animals</b>				
Eastda	189	56.8	56.8	56.8
Suothd	144	43.2	43.2	100.0
<b>Grazing type</b>				



Indoor		333	100.0	100.0	100.0
Outdoor		0			

**Water source**

Tap		333	100.0	100.0	100.0
River		0			

**Snail presence**

No		333	100.0	100.0	100.0
Yes		0			

**Water bodies**

No		333	100.0	100.0	100.0
Yes		0			

**Vegetation**

No		333	100.0	100.0	100.0
Yes		0			

**Table 3.2 continue**

**Knowledge of the disease**

No		18	5.4	5.4	5.4
Yes		315	94.6	94.6	100.0

**Manure disposal**

No		333	100.0	100.0	100.0
Yes		0			0

**Faciolaiasis**

Yes		314	94.3	94.3	94.3
No		19	5.7	6.3	100.0

+ve <b>Schistosomiasis</b>	332	99.7	99.7	99.7
+ ve	1	.3	.3	100.0
-				
ve <b>Other disease</b>	331	99.4	99.4	99.4
+ ve	2	.6	.6	100.0
-ve <b>Treatment of disease</b>	277	83.2	83.2	83.2
No	56	16.8	16.8	100.0
Yes				
.				

**Table 3.3:** Summary of cross tabulation for the rate of paramphistomiasis in each category of the potential risk factors in 333 cattle examined by fecal sedimentation

method at Ganawa slaughterhouse, Omdurman locality, Khartoum state, Sudan.

<b>Risk Factors</b>	<b>No. inspected</b>	<b>No. affected (%)</b>
<b>Age</b>		
Young( ≤3)	11	4 (36.4)
adlut( >3)	322	69(21.4)
<b>Sex</b>		
Female	333	73(21.9)
Male	0	
<b>Breed</b>		
Local	333	73(21.9)
0		
<b>Bodycondition</b>		
Good	6	5(83.3)
Poor	327	68(20.8)
<b>Suorce of animal</b>		
East	189	42(22.2)
darfur	144	31(21.5)
West		
darfur		
<b>Grazing type</b>		
Indoor	333	73(21.9)
0		
Outdoor		
<b>Water source</b>		
	333	73(21.9)

Tap		0	
River			
<b>Snial presence</b>	No	333	73(21.9)
	Y	0	
es			
<b>Water bodies</b>			
	No	333	73(21.9)
		0	
Yes			
<b>Vegetation</b>			
	No	333	73(21.9)
		0	
Yes			
<b>Table 3.3 continue</b>			
<b>Knowledge</b>			
		18	2(11.1)
No		315	71(22.5)
Yes			
<b>Manure disposal</b>			
	N	333	73(21.9)
o		0	
	Yes		
<b>Fasciolaiasis</b>			
		314	64(20.3)
-ve		19	9(47.4)
+ve			
<b>Schistosomaiasis</b>			
		332	73(21.5)
-ve		1	0
	+		
ve			
<b>Other disease</b>			
	-	331	72(21.8)

ve		2	1(50)
	+		
ve			
<b>Treatment</b>			
	N	277	69(24.9)
o		56	4(7.1)
	Yes		

**Table 3.4:** Summary univariate analysis for association between paramphistomiasis and potential risk factors by fecal sedimentation method using the Chi-square test in 333 cattle examined at Ganawa slaughterhouse, Omdurman locality Khartoum state, Sudan.

<b>Risk Factors</b>	<b>No. inspected</b>	<b>No. affected (%)</b>	<b>d. f</b>	<b>X2val ue</b>	<b>p-value</b>
---------------------	----------------------	-------------------------	-------------	-----------------	----------------

<b>Age</b>	Young(≤ 3)	11 322	4 (36.4) 69(21.4)	1	1.386	.239*
	adlut(> 3)					
<b>Sex</b>	Male	333	73(21.9)	1	Sex is a constant	–
	Femal	0				
<b>Breed</b>	Local	333	73(21.9)	1	Breed is a constant	–
	Cross	0				
<b>Bodycondition</b>	Poor	6 327	5(83.3) 68(20.8)	1	13.463	.000*
	Good					
<b>Suorce of animal</b>	Eastdarfu	189	42(22.2)	1	.023	.879
	Suothdarfu	144	31(21.5)			
<b>Grazing type</b>	Indoor	333	73(21.9)	1	GT is a constant	–
	Outdoor	0				
<b>Water source</b>	Tap	333	73(21.9)	1	WS is a constant	–
	River	0				
<b>Snail presence</b>		333	73(21.9)		SP is a constant	–

	N	0		1	nt	
o	Yes					
<b>Water bodies</b>	N	333	73(21.9)	1	WB is a constant	-
o	Yes					
<b>Vegetation</b>	N	333	73(21.9)	1	Vegetation is a constant	-
o	Yes					
<b>Knowledge</b>	N	18	2(11.1)	1	1.299	.254
o	Yes	315	71(22.5)			
<b>Manure disposal</b>		333	73(21.9)	1	Manure disposal is a constant	-
No	Yes					
<b>Fasciolaiasis</b>		314	64(20.4)	1		.006*
-ve		19	9(47.4)		7.623	
+ve						
<b>Schistosomiasis</b>		332	73(21.9)	1		.596
-ve		1	0		.282	
+ve						
+ve						

<b>Other disease</b>		331	72(21.8)	1	.927	.336
-ve		2	1(50)			
<b>ve</b>						
-ve						
+ve						
<b>Treatment</b>		277	69(24.9)	1		
No		56	4(7.1)		8.591	.003*
es	Y					

• Significant value

**Table 3.5:** multivariate analysis for The association between paramphistomiasis and potential risk factors in 333 cattle examined by fecal sedimentation method .at Ganawa slaughterhouse, Omdurman locality, Khartuom state, Sudan.

<b>Risk Factors</b>	<b>No. inspected (%)</b>	<b>No. affected (%)</b>	<b>Exp(B)</b>	<b>p-value</b>	<b>95.0%C.I</b>	
					<b>Low</b>	<b>High</b>
<b>Age</b>					.223	4.51
Young( $\leq 3$ )	11	4(36.4)	1.003	0.239		5



adlut(>3	322	69(21.4)	Ref			
)						
<b>Body condition</b>	6	5(83.3)	.056	.000	.006	.513
Poor	327	68(20.8)	Ref			
Good						
<b>Fasciolaiasis</b>						
-ve	314	64(20.4)	Ref	.006	.119	.808
+ve	19	9(47.4)	.310			
<b>Treatment</b>						
No	277	69(24.9)	3.629	.003	1.25	10.4
Yes	56	4(7.1)	Ref		7	76

## **Chapter Four Discussion**

Results of the present study have increased knowledge on the epidemiology of paramphistomiasis in cattle at Ganawa slaughterhouse in Khartoum state of the Sudan, investigated by using meat inspection test, fecal sedimentation method and questionnaires. Meat inspection and fecal sedimentation

method showed that the prevalence rate of paramphistomiasis was considerably high in the study area. While few studies have been conducted on paramphistomiasis in cattle in the Sudan.

Therefore, this study was conducted to estimate the prevalence rate of paramphistomiasis in cattle and to investigate potential risk factors associated with the occurrence of paramphistomiasis in Khartoum state.

In this study, the overall prevalence rate of egg of *paramphistomes* in cattle fecal samples collected from Ganawa slaughterhouse in Khartoum state were 21.9% (73/333) by both methods meat inspection test and fecal sedimentation method.

The results obtained from fecal sedimentation method in the present study was higher than the prevalence reported by Gad Alkareem(2012) in Sudan who reported a prevalence of 11.25% (18/160) in cattle, by Sanchis (2013) in Spain who reported a prevalence of 7%(56/803) , by Shabih(2006) in India, who reported a prevalence of 1.99% (7/351) , by P. Di ´az(2006) in Spain who reported a seroprevalence of 10.1% (53/524), by khan (2008) in India who reported a prevalence of 7.83% (188/2400), and by Shabih (2006) in India who reported a prevalence of 3.4% (22/651). However the prevalence reported in the present study was lower than the prevalence reported in Sudan of 29.5% (46/156) by Motasim (2014), bangladesh of 53.1% (191/360) by paul (2011). In

Ethiopia of 45.8% (176/384) by Yeneneh(2012).In Ethiopia of 44.23% (46/104) by fromsa (2011) and by Krishna (2013) in Bangladesh who reported aseroprevalence of 30% (32/107). This could be due to the differences in the tested sample size (n), practicing of traditional communal grazing 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. Very few studies in the Sudan have addressed risk factors associated with positivity to paraphistomiasis in cattle.

In the current study, univariate analysis using Chi -square, with a confidence interval of 95% at a *p-value* of  $\leq 0.25$  was used to identify potential risk factors associated with both methods meat inspection test and fecal sedimentation method positivity for paraphistomiasis in cattle. Significant risk factors associated with being meat inspection and fecal sedimentation method positive in the univariate analysis were age ( $X^2 = 1.386$ ,  $p = 0.239$ ), Body condition( $X^2 =$

13.463,  $p = 0.000$ ), Fasciolaiasis ( $X^2 = 7.623$ ,  $p = 0.006$ ) and Treatment of disease ( $X^2 = 8.591$ ,  $p = 0.003$ ).

The multivariate analysis, using logistic regression, with a confidence interval of 95% and a  $p$ -value of  $\leq 0.05$  was used to assess the association between identified significant risk factors in the univariate analysis in combination towards a positive meat inspection test and fecal sedimentation method status for paramphistomiasis in cattle. However, some potential risk factors which were regarded to be important with  $p$ -value  $\leq 0.25$  in the univariate analysis were also entered into the multivariate analysis. This analysis showed an association between being meat inspection test and fecal sedimentation method positive status for paramphistomiasis in cattle were age (Exp (B) = 1.003), body condition (Exp (B) = .056), fasciolaiasis (Exp (B) = .310) and treatment (Exp(B) = 3.629).

The positive association of body condition with meat inspection test and fecal sedimentation method paramphistomiasis positivity in cattle is in agreement with the findings by fromsa (2011) and Motasim(2014).

This positive association of body condition as risk factor could be explained by the fact that the fluke causes high protein losses in ruminant also the emaciated animal have lower resistance to fluke than cattle with a good body condition. The positive association of age, older animals especially cattle, seem to acquire immunity to the infection

by FAO (2009).The positive association of fasciolaiasis, any animal with fasciolaisis is at risk to the infection more than ones with out. The positive association of treatment, any animal didn't use prophylaxis drugs of the disease is at risk to the infection more than animals used of treatment of the disease, (Hugh-Jones ME, *et al.*, 2008).

### **Conclusion:**

From the results of this study, it can be concluded that cattle paramphistomiasis according to meat inspection diagnosis is prevailing in Ganawa slaughterhouse of Khartoum state at high prevalence rate by fecal sedimentation method (21.9%). Based on the results of this study, the risk factors associated with paramphistomiasis in cattle were: age, body condition, fasciolaiasis and treatment by both methods, Fecal sedimentation method and rumen dissection.

### **Recommendations:**

The study shows the need for:

- 1- More studies on potential risk factors that enhance the spread and transmission of paramphistomiasis in cattle in the Sudan.
- 2- Enforcement of legislation that will put end to backyard and road side slaughtering practices.
- 3- Extension and communication programs should be implemented to enable cattle and other livestock owners to understand the importance of the disease.
- 4- Integrated control and eradication program should immediately be launched as recommended by OIE.
- 5- The scheme of initiation of a regional network for surveillance, control and eradication of this important disease in the surrounding Africa countries.

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## **Appendices**

### **Questionnaire:**

Investigation of paramphistomiasis in Khartoum State.

Conducted by: The preventive medicine Department of Sudan University of science and technology, Faculty of veterinary medicine.

Locality \_\_\_\_\_ date \_\_\_\_\_

Animal 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:**

Poor ( )                      Good ( )

**5- Previous history of the disease:**

Yes ( )                      No ( )

**Comment:**

.....  
.....  
.....  
.....

**2-Management risk factors:**

**6- Source of animal:**

East darfuor ( )      South darfuor ( )

**7-Grazing type:**

Indoor ( )      Outdoor ( )

**8-water source:**

Tap ( )      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-Schistosoma:**

Positive ( )      Negative ( )

**16- Other disease:**

Positive ( )      Negative ( )

**17-Treatment of disease:**

Yes ( )      No ( )

**Comment:**

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