بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Prevalence of Sheep Haemonchosis in North Kordofan State, Sudan

نسبه الإصابة لمرض الهلاع في الضأن بولاية شمال كردفان – السودان

A thesis Submitted to the College of Graduate Studies in

Partial Fulfillment of the Requirements for the Degree of

Master in Preventive Veterinary Medicine

By

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April 2017

الآية

قَالَ تَعَالَىٰ:

﴿ ٱقۡرَأۡ بِٱسۡمِ رَبِّكَ ٱلَّذِي خَلَقَ ١ خَلَقَ ٱلۡإِنسَٰنَ مِنۡ عَلَقٍ ٢ ٱقۡرَأۡ وَرَبُّكَ ٱلۡأَكۡرَمُ ٣

ٱلَّذِي عَلَّمَ بِٱلۡقَلَمِ ٤ عَلَّمَ ٱلۡإِنسَٰنَ مَا لَمۡ يَعۡلَمۡ ٥ ﴾

العلق: ١ ـ ٥

Dedication

To my lovely father

To my kind unfailing support mother

To my brothers and my sisters

Acknowledgements

Firstly, praise to Almighty Allah for giving me the strength and stamina to finish this work. With a great touch of pleasure and gratitude, I would like to express thanks to my supervisor, Prof: Siham Elias Suliman or her advice, direction and continuous interest and constructive criticism in reviewing the dissertation.

My thanks extend to EL-Obeid slaughterhouse staff for assistance during the period of sample collection. Thanks to Veterinary Research Institute (VRI) ,El-Obeid which allowed me the chance to work in its labs , and My heartfelt thanks to Prof. Edris Ahmed Yagoub

My appreciation is extended to all who helped me in this study especially my colleagues Dr. Muzamil Omer .

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Abstract

A cross sectional study was conducted from September to November 2016 for determination of sheep haemonchosis and investigation of some associated potential Risk Factors in EL-Obeid slaughter house, North Kordofan, Sudan. A total of 355 fecal samples from Sheep were collected and examined using direct flotation test. The result indicated that natural Haemonchus contortus infection was prevalent among Sudanese sheep at EL-Obeid slaughter house with an overall prevalence 31.3%.

The following risk factors showed association with sheep haemonchosis in the univariate analysis under significant level of P-value $\leq$ 0.25: sex (P-value= 0.003), breed (P-value =0.000), body condition (P-value= 0.000). Using multivariate analysis to determine possible significant association between haemonchosis and potential risk factors, the result showed that there was significant association with all of the investigated risk factors.

It can be concluded that this parasite was prevalent in a high percentage in EL-Obeid slaughter house. Therefore, the use of anthelmintics by sheep farmers from period to period is recommended as a prophylactic measure.

ملخص البحث

أجريت دراسة مقطعية من سبتمبر إلى نوفمبر 2016 لتحديد معدل انتشار مرض الهلاع الضان والتحقيق في بعض عوامل الخطر المحتملة المرتبطة بها في مسلخ الأبيض في شمال كرفان بالسودان. تم جمع 355 عينة براز من الضان وفحصها باستخدام اختبار التعويم المباشر. وأظهرت النتائج أن عدوى هيمونشوس الطبيعية كانت منتشرة بين الأغنام السودانية في مسلخ الابيض بنسبة انتشار 31.3٪.

وأظهرت عوامل الخطر التالية الارتباط مع مرض الهلاع في تحليل وحيد المتغير تحت مستوى كبير من قيمة $P \leq 0.25$: الجنس (P- قيمة = 0.003)، سلالة (P- قيمة = 0.000)، حالة الجسم (P- قيمة = 0.000) . باستخدام التحليل متعدد المتغيرات لتحديد احتمال وجود ارتباط كبير بين مرض الهلاع وعوامل الخطر المحتملة، وأظهرت النتيجة أن هناك ارتباط كبير مع جميع عوامل الخطر التحقيق.

ويمكن أن نستنتج أن هذا الطفيلي كان سائدا في نسبة عالية في مسلخ الابيض . لذلك، يوصى باستخدام مضادات الديدان من قبل مزارعي الضان من فترة إلى أخرى كإجراء وقائي.

Introduction

Haemonchus contortus of the most important abomasal worms of sheep which is known as “red stomach worm” or “wire worm” of small ruminants. The parasite is pathogenic and also cause economic loss in sheep (Hansen , Perry ,1990) . The parasite most commonly found in sheep and goat but *Haemonchus placei* is the usually located in cattle and even so cross infection may occur when small ruminants and cattle graze together but the infestations are usually of less severity (Radostits *et al*.,2006).

The parasite found in abomasum of sheep and goat causes blood loss resulting in decrease in erythrocytes, lymphocytes, hemoglobin, packed cell volume, body weight, and wool growth. The abomasal nematode, which is particularly important and causes severe anemia and death in severely infected animals, identified haemonchosis as one of the top ten constrains to sheep and goat rearing in east Africa(Perry *et al.*,2002).

The first and second stages of larvae are free-living organism and the host ingests the third stage larvae in which starting the infection. Adults of the parasite are found on the surface of the mucosa (the lining of the stomach). Both the larvae (L4) and the adults of *Haemonchus* species suck blood. A thousand *Haemonchus* species of adult can suck 50 mL of blood/day causing severe anemia. A heavy infestation (20,000–30,000 worms) can kill sheep very quickly. All ages of sheep are susceptible to parasite infection but lambs are more susceptible than adults (Shapiro,2005).

The cardinal sign of haemonchosis is pallor of the skin and mucous membranes. A hematocrit reading of less than 15% is always accompanied by extreme weakness and shortness of breath and warrants a grave prognosis; less of plasma protein results in anasarca frequently manifested externally as a submaxillary edema (bottle jaw). The appetite typically remains good and, in acute outbreaks, affected animals may not lose appreciable weight. Feces are well formed, diarrhea occurring only in infections complicated by the presence of such species as *Trichostrongylus* species and *Cooperia* species. Lambs are the most seriously affected members of the flock, but older sheep under stress also may have fatal anemia (Bowman, 2003).

Objectives :

1- To estimate the prevalence of sheep haemonchosis in EL-Obeid slaughter house, north kordofan.
2- To investigate some potential risk factors associated with sheep haemonchosis in north kordofan.

CHPTER ONE

Literature Review

haemonchosis is a serious parasitic disease of domestic and wild animals in the tropics caused by the genus *Haemonchus* (Fabiyi ,1987) . Fayza *et al* . (2003) reported that *haemonchus contortus* the species that most commonly infects sheep and goats and to a lesser extent bovine and cameline species .

1.1.1.Etiology: *haemonchus contortus* (Francisco *et al*., 2007).

1.1.2.Classification

Kingdom: Animalia.

Phylum : Nemathelmintes.

Class: Secernetea.

Order: Strongylida.

Suborder: Trichostrongylina.

Superfamily: Trichostrongyloidea.

Family: Haemonchidae.

Subfamily: Haemonchinae.

Genus: *Haemonchus.*

Species: *H. cotortus.* (Francisco *et al*., 2007).

1.1.3.Morphology:

Eseta (2004) found that an adult parasite measures about 25 to 34 mm long, the male being shorter than the female which measure about 19 to Twenty two mm, the morphological characteristics of *Haemonchus contortus* are a mouth capsule with a single dorsal lancet and two prominent cervical papillae in the esophageal area .The male parasite is characterized by its copulatory bursa formed of two large lateral lobes (figure 1.1) and a small asymmetrically positioned dorsal lobe. Together with the two chitinous spicules, which are inserted in the female genital opening during copulation, this part of the worm is important for identification.

The females have a reddish digestive tube filled with ingested blood, spirally surrounded by two white genital cords (ovaries). They have asharply pointed slender tail and a vulva(Figure 1.2) with or without anterior vulval flap (Eseta, 2004). Easily visible to the naked eye and the female oviduct is visible as a white stripe around the red blood –filled intestine giving a barber pole appearance (figure 1.3) .

Jones *et al* .(2010) reported that eggs are typical of the *Trichostrongyloidia* Super family . The genus is among the largest in the super family ranging from 10-30mm in length. In fresh specimens the worms can be easily seen due to their bright red color and considerable size (figure 1.4) .

Figure 1.1: *Haemonchus* – male Bursal lobes (Eseta, 2004).

Figure 1.2: *Haemonchus* - female vulva area(Eseta, 2004) .

Figure 1.3: Blood in the gut of the parasite and the oviduct around the gut giving the characteristic barber's pole appearance(Terefe *et al*., 2005)

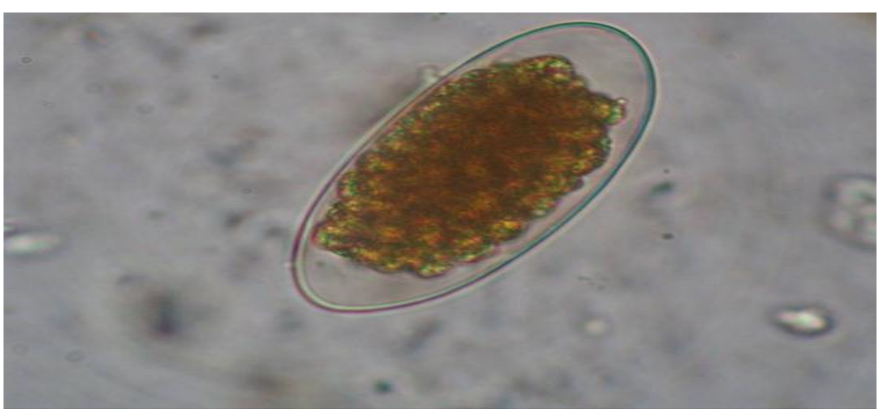

Figure 1.4: Egg of *Haemonchus* spp (Mostafa *et al*,.2013).

1.1.4. Life cycle of *Haemonchus contortus*:

Gastrointestinal nematodes (GIN) globally occur in sheep and goats. Depending on the local climatic conditions, different GIN species regionally predominate. They all have direct life cycles (figure. 5) , which are similar in all species and enable the worms to be readily transmissible in livestock. For each of them the prepatent period is approximately 20 days. Adult worms live in the abomasum and the small intestine (Scheuerle, 2009).

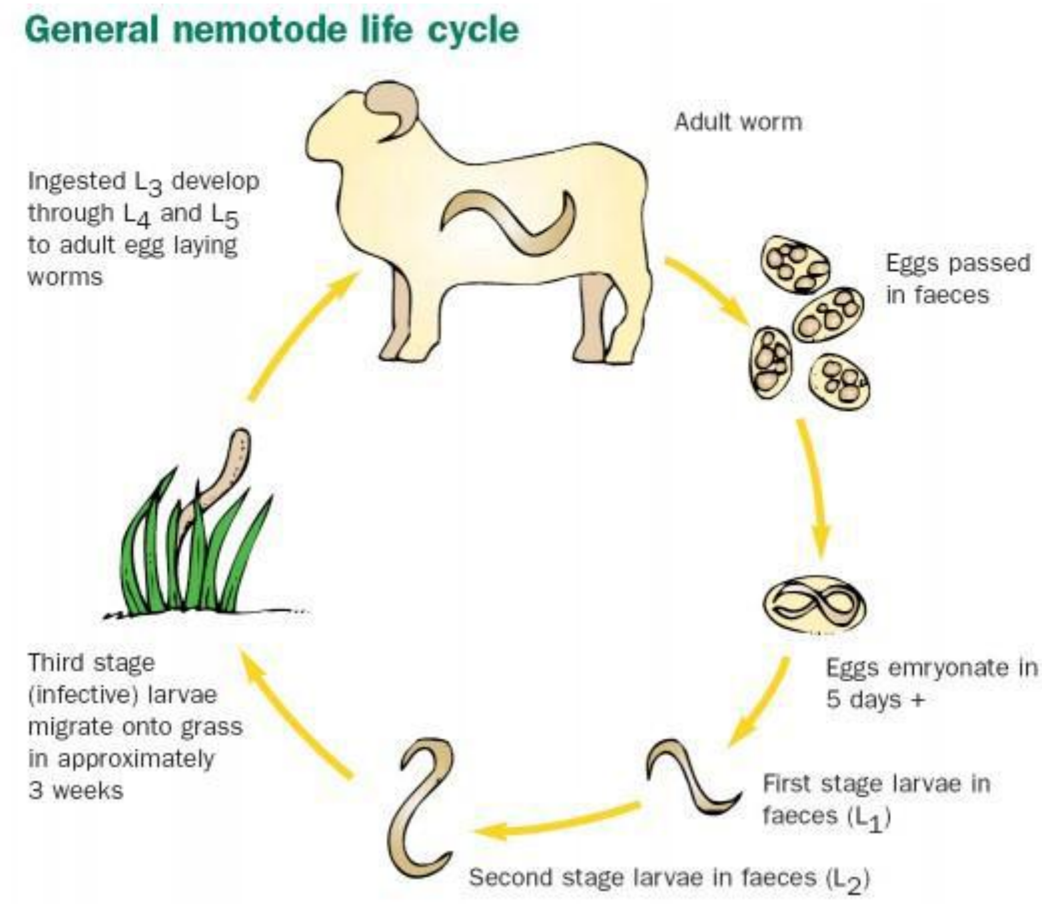

figure 1.5: General nematode life cycle (Scheuerle, 2009).

Brown (2011) found that the worms occur in the abomasum or four stomach of sheep and goats. They are up to 3 cm long. Female worms have a red and white striped appearance, hence the name 'barbers pole' The life cycle is typical of roundworms of sheep. Adult worms lay eggs which pass out in the faeces of the host. Barbers pole worms are the highest egg producers of all sheep worms . The life cycle of *H. contortus* consists of free-living stages on the pasture and parasitic stages within the host's abomasum (Terefe *et al*., 2005).

In these cycles, adult female parasites in the GI tract produce eggs that are passed out with the faeces of the animal .Development occurs within the faecal mass, the eggs embryonate and hatch into first-stage larvae (L1), which in turn moult into second-stage larvae , shedding their protective cuticle in the process. During this time the larvae feed on bacteria. The L2 moult into third-stage larvae (L3), but retain the cuticle from the previous moult. The L3 constitute the infective stage, and these migrate onto surrounding vegetation where they become available for ingestion by grazing sheep and goats (Sissay, 2007). Following ingestion, the L3 larvae pass to the abomasum or intestine, where they ex-sheath. The L3 of the worms penetrate the epithelial layer of the mucus membrane (*Haemonchus* and *Trichostrongylus*) or enter the gastric glands.

After ingestion, the larvae ex-sheath in the rumen and migrate to abomasum. The actual stimuli to ex-sheathment are unknown but are thought to be dissolved carbon dioxide and/or un dissociated carbonic acid in the gut .The parasitic exsheathed L3 migrate to the abomasum and become closely associated with the mucosa, where the third moult occurs and the fourth stage (L4) larvae emerge. The L4 is able to feed once the L3 sheath is lost and, just before the time of the fourth moult, the piercing lancet which enables the larvae to penetrate the surface of the abomasal mucosa develops. Feeding commences and is soon followed by the fourth moult to the fifth or pre-adult stage. After further feeding, the fifth stage larvae mature into adult worms which are to be found moving freely on the surface of the mucosa. Differentiation into male and female begins around the time of the fourth moult. They reach maturity in 15 days, the first eggs appearing in the feces of the host about 15 days after infection (Rugutt,1999).

Sissry (2007) reported that in normal development, the L3 moult within 2–3 days to become fourth-stage larvae (L4), which remain in the mucous membrane or in the gastric glands for a further 10 to 14 days. Finally, the L4 emerge and moult to become young adult parasites. The time between ingestion of L3 and the parasite becoming mature adults (refered to as the prepatent period) varies between parasite species, but generally 3 to 5 weeks .

Amaradsa *et al.* (2010) reported that the *H. contortus* infective larvae can survive beyond 21 days in the soil and infest pasture grasses when the climatic conditions are favourable . Adult worms only survive for several months in the host (Paddock, 2011). The free-living stages of Parasite are not as tolerant to unfavorable climatic conditions (cold, but particularly dry) as the other important nematode parasites of sheep (Donald, 1968; Waller and Donald, 1970). The highest biotic potential and pathogenicity of this parasite ensure that it is a major problem in the humid tropics and subtropics (Anon, 1991; Waller *et al.*, 1996; Chandrawathani *et al.*, 1999; Anon,2001). However, as Crofton *et al*. (1965) postulated several decades ago, *H.contortus*, in common with other nematode parasites of livestock, exhibits considerable ecological and biological plasticity to overcome unfavourable conditions either in the external, or host, environment (Waller *et al*., 2005)

1.1.5 : Hypobiosis phenomenon:

Soulsby (1982) repoted that an important phenomenon observed in the life cycle that has epidemiological implications is “arrested larval development” or “hypobiosis”.It is the “temporary cessation of development of a nematode at a particular point in its parasitic development” It is usually due to an unfavorable environmental stimulus, such as cold weather or dry conditions, received by the free-living L3 prior to ingestion and usually coincides with onset of winter or very dry conditions. Others factors and host factors are involved such as blood group ,breed of sheep may play role.

arrested development can occur in the gut of sheep or on pasture and ensures survival of the nematode under adverse climatic conditions, subsequent maturation of the larvae due to resumption of development known as the ‘spring rise’, when favorable conditions return in the spring, leads to a rapid rise in infection levels or fecal egg counts in the sheep (Hima .B,2003) .

1.2 Clinical manifestations and Signs:

Grtachew *et al* .(2007) found that haemonchosis in sheep may be classified as hyper acute, acute, or chronic. In the hyper acute form, death may occur within one week of heavy infection without significant signs. This form of the disease is very rare and appears only in highly susceptible lambs. The acute form is characterized by severe anaemia accompanied by oedema (“bottle jaw”). Anaemia is also characteristic of the chronic infection, often of low worm burdens and is accompanied by progressive weight loss. The chronic form is the most commonly observed during natural infections. The lesions are associated with anaemia resulting from blood loss. With the exception of the L3, all other stages of development feed on blood. The parasite is known to produce calcium and a clotting factor binding substance known as calreticulin , enabling the parasite to feed easily on host blood.

1.3.Diagnosis:

1.3.1.Direct smear method:

Amount of feces were taken on a clean slide and 1-2 drops of water were mixed with it. All the debris was removed. Then a cover slip was placed carefully and slide was examined under microscope for the presence of *Haemonchus contortus* eggs (Qamar, 2009).

1.3.2.Modified Mc master technique :

Faecal egg counts were determined by the modified Mc master technique with saturated solution of sodium chloride as the floating medium In each case, 3g of faeces were mixed in 42ml of saturated solution of sodium chloride then the number of parasite eggs per g of faeces was obtained by multiplying the total number of eggs counted in the two squares of the counting chambers of the Mc master slide by the dilution factor of 50, *Haemonchus contortus* eggs present were identified using standard parasitological criteria (Qamar, 2009).

1.3.3 Identification of the Worm :

The abomasa were removed from their abdominal cavity and ligated the end opening. Then the abomasa were opened along their greater curvature and close visualization was made for the presence of adult *Haemonchus* parasite. The worms were collected in normal saline. Then the parasites were identified based on the characteristics given by Hansen and Perry,(1990)

1.3.4.Centrifugal flotation :

method's more time is saved and greater accuracy obtained a sample of faeces , 1-5 g , is well mixed with water (about 30-50 ml) and strained through a sieve (1 mm mesh) to remove coarse faecal material . the mixture is sedimented for 10-15 minutes on the bench , or by light ceterifugation for two or three occasion , until the supernatant is clear . the sediment is then mixed with a saturated solution of sugar , salt or zinc sulphate in a centrifuge tube (15-50 ml volume) and centrifuged for one or two minutes at 500 g, the eggs were floated to the surface and then touched with a cover slip, and then the cover slip was placed on a clean slide and examined using a compound microscope at 10 x 10 magnification (soulsby 1982).

1.3.5.Serological diagnosis:

lone *et a l* .(2012) found that detection of infection during prepotency, is of greater Importance from the clinical point of view, therefore a simplified, field oriented Dot-ELISA has been developed for the detection of parasite soluble antigen in goat /sheep sera. Dot-ELISA performed with immunoaffinity purified somatic antigen could detect infection as early as one week post infection during pre-patency.

1.3.6. Molecular diagnosis:

by using polymerase chain reaction specific primers and amplicon for each target cDNA, a primer SYBR green real-time PCR assay on a gene amplicon 5700 sequence detection system (applied biosystems) and based on known ovine gene sequences (β-actin, IFN-γ, TNF-α, IL-3, IL-4, IL-5, IL-10,IL-12p40). Oligonucleotides were designed to amplify a product with a size of 51 base pair, with a melting temperature (Tm) of 58-60°C. When the ovine gene sequence was not known (Eotaxin, IL-13), a consensus sequence was created, based on a minimum of three known sequences in other mammalian species. PCR amplification on reverse transcript RNA obtained from ovine peripheral blood mononuclear cells (PBMC) stimulated with concanavalin (A) (ConA, 10 μg/ml, 24 hours in a 5% CO2 and 37°C atmosphere) was then performed (Getachew *et al.*, 2007).

1.4: Pathological effects:

Macroscopic and microscopic finding:

Macroscopic finding showed Petechial hemorrhage detected in the abomasal mucosa, with extensive mucosal hemorrhage, inflammation and mucous secretions around lesions, paleness of internal organs were also seen .The abomasal contents were fluid and partially covered with free blood; the carcasses were paled and have generalized edema and fluid throughout of the body cavities secondary due to hypoproteinemia . Microscopic finding showed: mononuclear cells infiltration (lymphocytes, monocytes and plasma cells), prominent eosinophilic infiltration in mucous glands (Fayza *et al.*,2003; Tehrani *et al* .,2012).

1.5 Self-cure phenomenon:

The most frequent described protective immune response against the abomasal nematode *haemonchus contortus* in sheep is the self-cure reaction. The self-cure reaction was considered as first evidence of immune expulsion of gastrointestinal nematodes. Sheep infected with parasite when allowed to graze in contaminated pasture showed suppression of egg production within a few days. However, this suppression of eggs often accompanied by elimination of adult worms and by a strong epidemiological re-infections. Self-cure reaction was the most protective immune response against abomasal nematodes (Fayza *et al.*,2003). This reaction is dependent on antigens associated with the living larvae and which act locally. Both host and parasite genetic factors may influence the occurance of the self-cure reaction. Self-cure is accompanied by a transient rise in blood histamine, an increase in the complement- fixing antibody titer and intense mucosal oedema in the abomasums (Soulsby,1982).

1.6 Treatment and control of haemonchosis:

1.6.1 Treatment:

1.6.1.1. Broad-spectrum anthelmintic:

The broad-spectrum anthelmintic can be divided into three groups on the basis of chemical structure and mode of action. These groups are:

Group 1- BZ, Benzimidazole (BZ) ('white' drenches):

BZ, Benzimidazole is effective against all nematodes and is ovicidal although individual generic products may vary in efficacy against some nematode species. After administration, the BZ passes into the rumen, which acts as a reservoir, allowing gradual release into the bloodstream. BZs act by inhibiting tubulin activity in intestinal cells of nematodes or tegum Pental cells of cestodes, preventing uptake of glucose. The longer the time it stays in the animal the more effective it is. There is one BZ anthelmintic (triclabendazole), which is narrow spectrum (liver fluke only) and differs from all the other BZs in many respects - but is classed with them because of its chemical structure (Abbott *et al*.,2009).

Group 2 - LM, Levamisole (LM) ('yellow' drenches):

Abbott *et al* .(2009) reported that the imidazothiazoles (levamisole) and tetrahydropyrimidines (morantel - no longer on the market). These drugs are rapidly absorbed and excreted and most of the dose is lost from the system within 24 hours. Therefore, it is not essential to maintain high concentrations in the sheep for protracted periods. LMs act on the nerve ganglion of the parasite, causing paralysis. They are not ovicidal. The therapeutic safety index, compared to other anthelmintics is low. Animals given levamisole may be hyperactive for a few minutes. Toxic signs, due to a stimulant effect on nerve ganglia, may manifest as salivation, bradycardia, and muscular tremors and in extreme cases death from respiratory failure. Injectable levamisole may cause inflammation at the site of injection .

Group 3 - ML, Macrocyclic lactones (ML) ('clear' drenches):

Abbott *et al* .(2009) found that the avermectins (ivermectin / doramectin) and the milbemycins (moxidectin). These compounds are highly lipophilic and following administration are stored in fat tissue from where they are slowly released. They act on glutamate gated Cl-channels and γ-aminobutyric acid (GABA) neurotransmission sites in nematodes, blocking interneuronal stimulation of inhibitory motor neurones, leading to a flaccid paralysis .

1.6.1.2. Narrow spectrum anthelmintics:

Peregrine *et al* .(2010) reported that The substituted phenols (nitroxynil) and the salicylanilides (closantel, oxyclozanide) are narrow spectrum anthelmintics. They are effective only against trematodes and blood sucking nematodes (*Haemonchus* and *Fasciola*). They act by uncoupling oxidative phosphorylation at the mitochondrial level, reducing the availability of ATP, NADH, NADPH. In the host they bind to plasma protein, which increases the duration of activity against blood sucking parasites.

1.6.2 Some control strategy:

A) Vaccination:

Nayebzadeh *et al* .(2008) reported that the parasite gut provides a potential source of protective antigens. In fact, substantial protection can be induced against parasite by immunizing lambs or goat kids with protein fractions isolated from the gut of this parasite. Such proteins are often known as hidden antigens because they are not recognized serologically by sheep which have acquired immunity following infection. Vaccination with the hidden antigen H11, a membrane glycoprotein with microsomal aminopeptidase-like activity isolated from the intestinal brush border of adult parasite , is known to protect adult sheep and young lambs against haemonchosis. Substantial protection has also been achieved by immunizing sheep with a glycoprotein fraction isolated from the intestinal membranes of the parasite.

B) Ratio of stock classes:

Young or susceptible animals are generally responsible for the vast majority of pasture contamination on a farm. Therefore contamination rates and parasitic disease may be reduced simply by reducing the proportion of young or susceptible stock on a farm .This can be assisted by selling or removing young stock earlier, saving fewer replacements or changing the principle product of the operation, e.g. from lamb to beef. Obviously these sorts of decisions will be dictated largely by economic considerations. In a sheep finishing situation, the main aim is to minimize the larval challenge to the most vulnerable and economically sensitive class of stock, the naïve lamb pre- and post-weaning. Any reduction in lamb growth rate due to internal parasites reduces carcass weight and/or extends the time period from weaning to slaughter which in turn decreases lamb value; increases competition between finishing lambs and ewes (pre-joining) for late-summer pasture; and increases the total pasture consumption of lambs to a given carcass weight. In the case of goat farms, because all classes of animals tend to remain relatively susceptible to infection, reducing the proportion of susceptible stock will normally mean replacing a proportion of goat stock units with cattle (or less preferably adult sheep). Long term intensive farming of goats by themselves is unlikely to be viable due to difficulties in achieving adequate parasite control (Rattary, 2003).

C) Level of feeding:

Optimal levels of nutrition are essential in combating parasitism and achieving good levels of production in its presence for all classes of stock. Level of nutrition, especially protein nutrition, allows the animals to tolerate internal parasite infections and develop a good immune response. “Drenching is not a substitute for good feeding” and “There is no better anthelmintic than good quality green grass” .To optimize feeding levels, a knowledge of feed requirements and optimum pasture covers for susceptible classes of stock is essential. Grazing management decisions should aim at providing these, or if unachievable, high quality supplements should be fed. Good levels of feeding of pregnant and early lactating ewes, in particular multiple bearing ewes and poor conditioned ewes, will help prevent the temporary breakdown in their immunity and the perparturient rise in faecal egg counts. This will result in lower levels of pasture contamination than otherwise would have been the case (Rattary, 2003).

1.7 Epidemiology:

1.7.1 Geographical distribution:

Waller and Chandrawathani .(2005) reported that the parasite infestation occur throughout the world; epidemiological studies describe the lower environmental limits for haemonchosis to occur in sheep, as being a mean monthly temperature of 18ºC and approximately 50 mm rainfall. Thus it has been generally recognized that parasite is a problem parasite restricted to the warm, wet countries where sheep and goats are raised. However, recent evidence shows that this parasite is apparently common even in northern Europe .

1.7.2 Prevalence of sheep haemonchosis:

In a study was carried out in Sudan at Omdurman abattoir one thousand and two hundred abomasal samples from sheep were examined for detection of the prevalence rate of *Haemonchus contortus* .The results of this study indicated that haemonchosis was widely spread among sheep. The prevalence rate of parasite displayed by this study was 32% (Fayza *et al.*, 2003).

A cross sectional study was conducted from March to May 2015 for determination of ovine haemonchosis and investigation of associated potential Risk Factors in Khartoum State, Sudan. A total of 170 fecal samples from Sheep were collected and examined using Direct smear and Centrifugal flotation test and then culture of faces. The result indicated that natural parasite infection was prevalent among Sudanese sheep at Khartoum State with an overall prevalence 6.5% (Mona,2015).

In epidemiological studies carried out at slaughterhouses, livestock farms and veterinary hospitals under different climatic condition existing in Punja province, the prevalence in slaughtered animals, veterinary hospitals and at livestock farms was 36.07%, 40.01% & 38.45% respectively. The highest district wise prevalence was noted at Gujranwala (40.67%), followed by Sheikhupura (39.5%) then Kasur (37.97%) and the lowest at Lahore (28.94%). As regards the season wise prevalence the highest prevalence was noted during summer (43.95%) followed by autumn (38.75%) whereas the lowest (28.8%) during winter. It was revealed that prevalence was higher(40.31%) in animals below 9months of age than in above 9 months of age(33.08%). Animals of either sex were equally affected (Qamar *et al*., 2009).

A survey study was carried out from December 2010 to November 2011 in order to establish the epidemiology of *Haemonchus contortus* infections in small ruminants of Benin. A total of 756 abomasum's, collected from randomly selected goats and sheep from all regions of Benin has been examined. An examination of the conjunctiva's colour has been associated with parasitic diagnosis to assess the degree of anaemia in animals. The study disclosed an endemic evolution of haemonchosis. The overall prevalence was of 55.56% with a mean burden of 175 worms per infested animal. No Significant influence could be attributed to host's species or age. The season has been a significant variation factor ($p<0.001$). The prevalence of haemonchosis was higher in wet seasons (79.41%) than in dry (36.06 %). The worm's burden was also higher in rainy seasons than dry. Elsewhere, a Strong correlation ($p<0.001$) was found between the conjunctiva colour and the worm burden but with a reverse influence of the season. In rainy seasons, degrees of anaemia have been low even though worm burdens were high. Inversely, moderate worm burdens induced detectable anemia during dry Seasons (Attindehou *et al*., 2012).

In a study was carried out at government research Centre for conservation of Sahiwal cattle Jehangirabad, district Khanewal from February 2007 to June 2007, to investigate the overall prevalence of *Haemonchus contortus* in sheep. The present study revealed that the parasite had an overall prevalence of (77.7%). The males showed significantly ($P<0.05$) higher prevalence (84.6%) as compared to females (72.1%). Maximum prevalence (100%) was recorded in age group of 186-205 months and minimum (50%) in the age group of 146-1650 months showing the statistical significance ($P<0.05$). Maximum prevalence (100%) was recorded in weight group of 72-78 and 79-85 kg, while weight group of 58-64 kg had minimum prevalence (50%) with statistical significance($P<0.05$).The prevalence was statistically different ($P<0.05$) in different breeds of sheep; Awassi was more susceptible showing higher prevalence (93.3%) followed by Lohi (85.9%) and Hisardale (74.4%) (Tasawar *et al*., 2010).

In a study was conducted to determine the prevalence of *Haemonchus contortus* in slaughtered sheep and goats at Multan abattoir. A total of 4740 animals were slaughtered and examined from 21 January 2007 to 20 February 2007 in Multan Abattoir. In case of sheep, 793 out of 2133 were positive and prevalence of *Haemonchus contortus* infestation was 37.18% while 811 out of 2607 (31-10%) goats were positive. Sex wise prevalence of *Haemonchus contortus* in sheep was 34.11% (291/853) in male and 39.22% (502/1280) in female while in goats prevalence in male was 29.91% (312/1043) and in female was 31.90% (499/1564) (Raza *et al*., 2009).

A cross sectional study was performed with an attempt to determine the prevalence and associated risk factors of haemonchosis in randomly selected slaughtered sheep and goats in restaurants and hotels in Gondar town, Amhara region, northwest Ethiopia from November 2011 to April 2012. A total of 384 animals (335 sheep and 49 goats) were examined. Overall prevalence was 80.21%. The specific prevalence of *Haemonchus. contortus* infection was 81.2% and 73.5% in sheep and goats respectively. The difference in infection rates between the Two species was not statistically significant ($X2= 1.607$, $p>0.05$). The prevalence of haemonchosis in males and females was 80.9% and 77%, respectively but, the difference is not statistically significant ($X\ 2= 0.583$, $p>0.05$).Relationship between body condition and haemonchosis in sheep and goats showed no statistical difference ($X2=1.727$, $p>0.05$) between medium and good body conditioned animals. In the present study, a high infection rate with *Haemonchus contortus* was observed in both sheep and goats during the study period affecting health of those animals and appropriate control measure should be instituted (Fentahun and Girja ,2012).

A cross sectional study was conducted in Woreda Alameta, southern Tigrary to estimate the prevalence of haemonchosis in small ruminants in four different hotels of Alameta twon from

November 2011 to March 2012. During the study period , 613 abomasum of small ruminants, 355 sheep and 258 goats, were examined. The overall prevalence in this study was 38.6%, with a prevalence of 22.8% , and 15.8% were recorded for sheep and goats respectively. The prevalence was compared with species, age, sex, origin, month and body condition of the animal. There was no statistically significant difference ($P < 0.05$) observed among risk factors of age , sex and species . However, there was statistically significant difference ($P > 0.05$) noticed among origin , months , and body condition of animals in relation to the parasite was recorded in animals with poor body condition (71.5%), followed by medium body condition (36.7%) and the lowest was recorded in animals having good body condition (19.5%). The highest prevalence was recorded the month of February (30.8%). Therefore , the epidemiological evidence of the present investigation showed that haemonchosis is considerably prevalent disease of small ruminants in the study area. Hence, strategic control methods and good management practice are recommended (Tsegabirhan *et al*., 2013).

CHAPTER TWO

Materials and Methods

2.1.1.Study area:

The study was carried out in North Kordofan State. Which lies in the arid and semi-arid zones between latitude 11.15-16.45º N and longitude 27-32.15º E. It borders the Northern state in the north, Northern and Southern Darfur states in the west, West and South Kordofan states in the south, and the White Nile and Khartoum states in the east. The city of El Obeid (capital of state)lies at latitudes 130 and13.150 north and longitudes 30.080 and 30.150 east. El Obeid population is estimated at 345126 inhabitants (CBS ,2011). Annual rainfall is concentrated in a single relatively short summer season during June to September and the region enjoys an annual rainfall of 0 to 500 mm.. Agriculture and livestock comprise about 70% of the economic activity in Kordofan (El-Hag *et al*., 2011).

A mixture of farming systems are practiced in the region including nomadic, sedentary and semi-sedentary animal production systems. Kabashi and Hamarri desert sheep, the main breeds raised in the region, are considered the best breeds for live sheep and the second-best breed for meat. The bulk of the Sudan's live sheep exports and meat for local consumption are from this region. In addition, most of the large sheep (average 35–45 kg live weight) and high-quality lambs purchased during the annual Hajj and Ramadan religious festivals originate from Kordofan region (ILRI,2009).

Figure 2.1:Map of study area North Kordofan (El-Hag *et al*., 2011)

2.1.2. El-Obeid Abattoir:

El-Obeid is capital city of North kordofan. This abattoir is located in the west of EL-Obeid, North kordofan State .It consists of administrative building, Veterinary Services Department, Maintenance Department . Cattle is slaughtered in the basement, goats and sheep on the top floor. The capacity of slaughter house is 200 head of sheep per day. The ante-mortem and post mortem examination are conducted by veterinarians. Fluids are disposed off through the sewage system and the solid parts through burning in the incinerator.

2.2. Type of study:

The study design was a cross sectional study which provided information on occurrence of a disease (Martin *et al*. , 1987). A Cross-sectional study was conducted at El-Obeid abattoir on three randomly selected days .These days selected were Sunday, Tuesday and Thursday .The animals in these days selected by systematic random sampling method. From each five animals one animal was selected for examination.

Prevalence of sheep haemonchosis was calculated by the following formula (Farooq *et al.*, 2012) :

$$\textbf{Prevalence Rate} = \frac{\textbf{No of sheep with haemonchosis}}{\textbf{Total No of sheep tested at a particular point in time}} \times 100$$

2.3. Sample size:

The sample size for determining the prevalence of haemonchosis in sheep in North Kordofan was calculated according to Thrufield ,(2007)and based on the following parameters: 95.0% level of confidence, ±5% desired level of precision and the expected prevalence of sheep haemonchosis was 36.4% (Mubarak,2014). By using the following formula:

$n = (1 .96)^2$ Pexp. (1 – Pexp.) d^2 Where:

n = required sample size Pexp = expected prevalence

d = desired absolute precision

The required sample size was found to be 355 animals.

2.4.Ante –mortem examination :

Regular visits were made by the investigator to conduct ante –mortem examination of slaughter animals. A total of 355 sheep were examined in the El-Obeid abattoir during the survey period which extended from September 2016 to November 2016 . During the ante mortem inspection, the age, sex, breed and body condition of each animals were determined . The age of animals was determined by incisors of animals teeth. Body condition of each individual animal was assessed and recorded depending on their body condition score, were ranked as poor or medium. Animal origin was difficult to know the exact origin of animals ,because the animals obtained from different markets.

2.5. Sample collection and laboratory diagnosis:

2.5.1.Sample collection:

during ante-mortem examination was performed a few hours before slaughtering from randomly selected sheep .Fecal samples 355 were collected per rectum and then transported to laboratory of Veterinary Research Institute (VRI) , in El-Obeid .

2.5.2.laboratory diagnosis:

2.5.2.1. Identification of the Worm:

During post mortem 12 abomasa of sheep were removed from their abdominal cavity and ligated at open ending. Then the abomasa were opened along their greater curvature and close visualization was made for the presence of adult *Haemonchus* parasite. The worms were collected in normal saline. Then the parasites were identified based on the characteristics given by Hansen and Perry,(1990). Then identified *Haemonchus* egg after crushing female worm.

2.5.2.2. Direct flotation test:

A sample of feces was weighed by using a pre calibrated teaspoon approximately 3g of faces were put into container, mixed with a saturated solution of sodium chloride (NaCl) and strained through a sieve (1 mm mesh) to remove coarse fecal material. the mixture was placed in a centrifuge tube (15-50 ml volume) , and then the cover slip was placed on top of a centrifuge tube for 10 minutes on the bench. the eggs were floated to the surface and then touched with a cover slip, and then the cover slip was placed on a clean slide and examined using a compound microscope at 10 x 10 magnification (Soulsby 1982)

2.6. Statistical analysis:

Frequency tables of the distribution according to the potential risk factors was constructed . Cross-tabulation of haemonchosis according to potential risk factors was made. Univarate analysis by the Chi-square test using statistical packets for Social Sciences (SPSS).Multivariate analysis by Logistic Regression models to perform risk factors significant at level ≤ 0.25 in the Univarate model. The significant level in the Multivariate analysis was be $\leq$ 0.05.

CHAPTER THREE

Results

A total of 355 sheep were tested by direct flotation test , 111 animals were found positive and 244 animals were found negative for sheep haemonchosis .Therefore, the overall prevalence of sheep haemonchosis in EL-Obeid slaughter house North kordofan was 31.3% (Table 3.1).

Table3.1: Distribution of *Haemonchus* infection among 355 sheep examined in EL-Obeid slaughter house:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	-ve	244	68.7	68.7	68.7
	+ve	111	31.3	31.3	100.0
	Total	355	100.0	100.0	

Sex of animal :

The total number of female examined was 216 animals. Among these, 80 animals were found infected and the rate of infection was 37%. The total number of males examined was 139 . Among these, 31 animals were found infected. The rate of infection was 22.3% (Table 3.2).

The Chi-square test, showed that there was significant association between *haemonchus contortus* infection (Table3.4) and sex of animal (p-value =0.003) .

Age of animals :

The result showed that age of animals, 165 of the sheep were less or equal to one years (young) and 190 of sheep were more than one years (old), (Table3.2). Among young animals 48 animals were found to be infected and the rate of infection within young animals was{ 29%}. However among adults 63 animals were found infected (Table 3.3).and the rate of infection was 33.1 %.

The Chi- square test showed no significant association between haemonchosis (Table3.4) and age of animals (p-value=0.410).

Breed of animal :

The results of study showed distribution of haemonchosis according to ecotype. All the ecotypes were local ecotypes, 127 Kabashi and 228 Hamarri , about 27 animals were positive from Kabashi and 87 animals were positive from Hamarri (Table3.2). The rate of infection was 21.2% in Kabashi breed and 38% in Hamarri breed (Table3.3).

The Chi- square test showed significant association between the infection and breed (p-value = 0.000), (Table3.4).

Body condition :

The body condition of animals and the presence of infection were investigated and 204 of sheep were found to be in medium condition, while 151 of sheep were found to be in poor condition (Table3.2). Among medium body condition 44 animals were found infected. The rate of infection within medium animals was 21.7%. However, 67 animal was found infected among poor animals. The rate of infection within poor animals was 44.3% (Table3.3).

The chi- square test showed highly significant association between the haemonchosis and body condition (p-value = 0.000), (Table3.4).

Results of multivariate analysis:

Three potential risk factors were found to be significantly (P-value < 0.25) associated with sheep haemonchosis in the univariate analysis (Table 3.4) entered to logistic regression; final Amodel, all factors were significantly (P-value < 0.05) associated with sheep haemonchosis in the multivariate analysis (table 3. 5).

Table3.2: Summary of frequency for potential risk factors of haemonchosis in 355 sheep examined at EL-Obeid slaughterhouse:

Risk factor	**Frequency**	**Relative frequency (%)**	**Cumulative frequency (%**
Sex :			
male	139	39.2	39.2
female	216	60.8	100.0
Age:			
young	165	46.5	46.5
Old	190	53.5	100.0
Breed:			
Kabashi	127	35.8	35.8
Hamarri	228	64.2	100.0
Body condition:			
Medium	204	57.5	57.5
Poor	151	42.5	100.0

Table3.3: Summary cross-tabulation of haemonchosis in332 sheep examined at EL-Obeid slaughterhouse .

Risk factors	**Animals tested**	**Animals affected**	**Rate of infection %**
Sex :			
male	139	31	22
female	216	80	37
Age:			
young	165	48	29
old	190	63	33
Breed:			
Kabashi	127	87	21
Hamarri	228	24	38
Body condition:			
medium	204	44	21
Poor	151	67	44

Table3.4: Summary of univariate analysis for potential risk factors of haemonchosis in 355 sheep examined at EL-Obeid slaughterhouse using the chi square test

Risk factors	**No. inspected**	**No. affected (%)**	**Df**	**X^2**	**p- value**
Sex : male female	 139 216	 31(22)% 80(37)%	1	8.544	.003*
Age: young old	 165 190	 48(29)% 63(33)%	1	.680	.410
Breed: Kabashi Hamarri	 127 228	 27(21)% 87(38)%	1	14.079	.000*
Body condition: medium Poor	 204 151	 44(21)% 67(44)	1	20.993	.000*

*Means significant value .p- value ≤0.25.

Table3.5: Multivariate analysis of haemonchosis and potential risk factors in 355 sheep examined at EL-Obeid slaughterhouse using logistic Regression:

Risk factors	Animals affected (%)	Exp(B)	95% Confidence Interval for Exp.(B)		p- value
			Lower	Upper	
Sex : male female	 31(22)% 80(37)%	 Ref 5.600	1.528	20.520	.009*
Breed Kabashi Hamarri	 27(21)% 87(38)%	 Ref .166	.049	.570	.004*
Body : medium Poor	 44(21)% 67(44)%	 Ref .313	.158	.623	.001*

*Means significant value .p- value ≤0.05.

CHAPTER FOUR

Discussion

In the present study the prevalence of disease was 31.3% in sheep in El-Obeid slaughter house, North kordofan, Sudan .The result is similar to another study carried out in Omdurman slaughter house ,Khartoum state, Sudan from where the rate of infection in sheep was 32% (fayza *etal*.,2003) . The prevalence of haemonchosis in this study is lower than the results in other studies which was 36.4% in North kordofan ,Sudan (Mubarak, 2013) .Also the study carried out in abattoir of Tulus locality in South Darfur state, Sudan which was 53.4% (Almalik *et al.*,2008) and another report in Omdurman Slaughter house which was 80% (Gagood *et al*.,1968).

On the other hand the prevalence of haemonchosis recorded during this study is higher than the results of Mohammed (2014), Mona (2015) and Mohammed *et al*. (2016) in Khartoum state (12.1% ,6.5% ,12.1%).Therefore ,this different between prevalence could be due to seasonal variation. The result of this study is much lower than the results in another studies in different countries which was ,72,5% in Ethiopia (Sissery , 2007) , 40.7% also in Ethiopia (lidya and Benhum, 2015) , 55.56% in Benin (Attindehou *et al.*, 2012) and 35,18% in Pakistan (Qamer *et al* . 2009).

The prevalence in this study is much higher than the result reported in Iran which was 9.3% (Tehrani *etal*., 2012).The differences in prevalence reported by these studies could be accounted on the basis of differential management practices (Barger, 1999; Lindqvist *et al*., 2001; Mandonnet *et al*., 2003), natural resistance (Pal and Qayyum 1992; Soulsby 2005; Chaudhry *et al*., 2007), drug treatment (Ali *et al*., 1997; Barnes *et al.*, 2001), and local geo-climatic factors (Gupta *et al*., 1987; Pal and Qayyum 1993; Chaudhry *et al*., 2007) and nutrition (Abbott et al., 1985; Preston and Allonby, 1987; Datta *et al*., 1999).

The prevalence of haemonchosis according to sex of animals was estimated in this study , the rate of infection in male was 22.3% and in female 37% .There was significant association (P-value =0,009). The highest rate of infection was found in female , this could be attributed to variation in sample size and more females to be sampled than males .The result is similar to anther result reported in Iran (Tenhrani *et al.*,2012) ,but this result is disagreed with study conducted in Sudan (Mona ,2015) and in Ethiopia (fentahum and Girja , 2012).

The result of present study showed the prevalence of heamonchosis with different age of animals that 33% in old and 29% in young .However there was no statistically significant association (P-value=0.410) between age of animals and haemonchosis .Old animals have higher rate of infection ,this may be due to variation in sample size . This finding in agreement with result of another study in Sudan (Mona, 2015).But disagreed with studies carried out in Sudan (Mubarak , 2013 ; Mohammed ,2014).

The prevalence of haemonchosis related to breed of animals was 38% in Hamarri and 13.9% in Kabashi breed .However , there was highly statistically significant variation in prevalence of haemonchosis among different breeds (P-value=0.000), Hamarri breed had higher rate of infection .This may be attributed to nature of pasture- grazing pattern and movement between these topographical location for pasture .This result in agreement with result carried out in Sudan (Mona, 2015).But this finding disagreed with study conducted in Sudan (Mohammed 2014; Mohammed *et al*. ,2016).

There was highly statistically significant variation in the prevalence of haemonchosis among different body condition ((P-value=0.000). The highest prevalence was seem in poor body condition while the lowest in medium body condition .The result of study is related to body condition of host seem to have influence on the prevalence of infection .Similar result had been reported in North kordofan (Mubarak,2013).But this result disagreed with study conducted in Khartoum State (Mona,2015).The highest infection rate recorded in poor body condition, may be due to the effect of heavy infection rate of *Haemonchus* parasite leading to significant weight loss.

Conclusion

The output of this study indicates , that the overall prevalence of haemonchosis was 31.3% . The presence of high rate of *haemonchus* parasite in the area was responsible for the loss of production in sheep. A high prevalence of infection was in female as compared to male. Old animals were highly effected as compared to young animals. A high prevalence of infection was in Hamarri breed as compared to Kabashi breed . Poor body condition more effected than medium body condition .

Recommendation

_Using of anthelmintics by sheep farmers from period to period is recommended as a prophylactic measure.

_The strategic deworming should be focused on poor and medium body condition sheep.

_ Improvement of husbandry practices is very important.

_Continuous surveillance of parasite in sheep is very essential .

_Further study on possible risk factor should be conducted .

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Appendix I

Frequency table for the distribution of infection among 355sheep examined at EL-Obeid slaughter house according to potential risk factors.

A. Frequency distribution of sex:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	139	39.2	39.2	39.2
	Female	216	60.8	60.8	100.0
	Total	355	100.0	100.0	

B. Frequency distribution of age:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Young	165	46.5	46.5	46.5
	Old	190	53.5	53.5	100.0
	Total	355	100.0	100.0	

c .Frequency distribution of breed:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Kabashi	127	35.8	35.8	35.8
	Hamarri	228	64.2	64.2	100.0
	Total	355	100.0	100.0	

D. Frequency distribution of body condition:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Medium	204	57.5	57.5	57.5
	Poor	151	42.5	42.5	100.0
	Total	355	100.0	100.0	

Appendix I I

Cross-tabulation for the distribution of infection among 355 sheep examined at EL-Obeid slaughter house according to potential risk factors investigated.

A. Sheep haemonchosis and sex cross-tabulation:

		Sex		
Count		**Male**	**Female**	**Total**
Result	_ve	108	136	244
	+ve	31	80	111
Total		139	216	355

B. Sheep haemonchosis and Age cross-tabulation:

		Age		
Count		**Young**	**Old**	**Total**
Result	_ve	117	127	244
	+ve	48	63	111
Total		165	190	355

C. Sheep haemonchosis and breed cross-tabulation:

Count		Breed		Total
		Kabashi	Hamarri	
Result	_ve	103	141	244
	+ve	24	87	111
Total		127	228	355

D. Sheep haemonchosis and body condition cross-tabulation:

Count		Body		Total
		Medium	Poor	
Result	_ve	160	84	244
	+ve	44	67	111
Total		204	151	355

Appendix III

Univarate analysis for the association of sheep haemonchosis in 355 sheep with potential risk factors using Chi square ($\chi2$) test.

A. Sex:

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	8.544	1	.003
Likelihood Ratio	8.780	1	.003
Linear-by-Linear Association	8.520	1	.004
N of Valid Cases	355		

B. Age:

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.680	1	.410
Likelihood Ratio	.681	1	.409
Linear-by-Linear Association	.678	1	.410
N of Valid Cases	355		

C. Breed :

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	14.079	1	.000
Likelihood Ratio	14.784	1	.000
Linear-by-Linear Association	14.040	1	.000
N of Valid Cases	355		

D. Body condition:

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	20.993	1	.000
Likelihood Ratio	20.929	1	.000
Linear-by-Linear Association	20.934	1	.000
N of Valid Cases	355		