

بسم الله الرحمن الرحيم

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**Prevalence and Risk Factors of Sheep Haemonchosis in Khartoum State,
Sudan**

نسبة الإصابة وعوامل الخطر لمرض الهلاع في الضأن بولاية

الخرطوم – السودان

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fulfillment of the Requirements for the Degree of Master of
Science in Preventive Medicine (M.P.V.M)**

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الآية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(وَلَوْ لَّا فَضَّلُ اللَّهُ عَلَيْكَ وَرَحْمَتُهُ لَهَمَّتْ طَائِفَةٌ مِنْهُمْ أَنْ يُضِلُّوكَ وَمَا يُضِلُّونَ إِلَّا أَنْفُسَهُمْ ۗ وَمَا يَضُرُّونَكَ مِنْ شَيْءٍ ۚ وَأَنْزَلَ اللَّهُ عَلَيْكَ الْكِتَابَ وَالْحِكْمَةَ وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ ۚ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا)

صدق الله العظيم

سورة النساء الآية (113)

Dedication

To my mother

To my sister's

To all who inspired me to face

The ups and downs of life.

and to the memory of my professor

dr. Hussein abdalla Hussein

And

Prof. Ahmed Ali Ismail

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Firstly, praise to Almighty Allah for giving me the strength and stamina to finish this work.

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Abstract

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 faeces.

The result indicated that natural *Haemonchus contortus* infection was prevalent among sudanese sheep at Khartoum State with an overall prevalence 6.5%. The following risk factors showed association with sheep haemonchosis in the univariate analysis under significant level of P-value ≤ 0.25 : bread (P-value=0.023), housing type (P-value =0.026), source of water (P-value=0.065), use of anthelmintics (p-value=0.002), owner knowledge (p-value=0.003), vegetation area (p-value=0.073), type of soil (P-value= 0.000) and localities (P-value= 0.032).

Using multivariate analysis to determine possible significant association between haemonchosis and potential risk factors, the result showed that there was no significant association with any of the investigated risk factors.

The highest prevalence of infection was in karary (18.2%), and East Nile (17.6%), then Bahri (12.5%), and Omdurman (2.7%). Whereas the lowest prevalence of infection was in Khartoum (0%) and Jabal al Awliya (0%) and um bada (0%).

ملخص البحث

أجريت دراسة مقطعية في الفترة من شهر مارس حتى مايو 2015 على عدد 170 رأس من الضأن في ولاية الخرطوم ؛ لتقدير معدل انتشار مرض هلاع الضأن في ولاية الخرطوم والتقصي حول عوامل الخطر المرتبطة به . تم تشخيص المرض بواسطة تحليل براز الحيوان لفحص بيض وزراعة يرقات الطفيل .

أظهرت الدراسة أن نسبة انتشار المرض في الضأن بولاية الخرطوم تعادل 6,5% في التحليل الفردي باستخدام إختبار الطفح لتحليل عوامل الخطر وجدت علاقة معنوية- تحت قيم معنوية أقل من أو يساوي 0.25 - بين حدوث المرض و كل من عوامل الخطر التالية : سلالة الحيوان (القيمة المعنوية = 0.023) ' نوع السكن (القيمة المعنوية = 0.026) ' مصدر المياه (القيمة المعنوية = 0.065) ' استخدام مضادات الديدان (القيمة المعنوية = 0.000) ' معرفة المالك (القيمة المعنوية = 0.003) ' منطقة الغطاء النباتي (القيمة المعنوية = 0.073) ' طبيعة الأرض (القيمة المعنوية = 0.000) المحليات (القيمة المعنوية = 0.032) .

باستخدام التحليل متعدد المتغيرات المتعدد لمعرفة درجة الارتباط بين مرض الهلاع وبين عوامل الخطر المحتملة ' أظهرت النتيجة أنه لم يكن هناك أي ارتباط معنوي مع أي من عوامل الخطر بمرض الهلاع في الضأن في ولاية الخرطوم .

كان أعلى معدل انتشار للعدوى في كرري (18.2%)، وشرق النيل (17.6%)، ثم بحري (12،5%)، وأم درمان (2.7%). في حين كان أدنى معدل انتشار للعدوى في الخرطوم (0%) وجبل الأولياء (0%) و أمبدة (0%) .

Introduction

Haemonchosis is one of the most serious parasitic disease of domestic and wild Animals. It cause great economic losses in sheep and goats especially in the tropic (Francisco *et al.*,2007; Chuadary *et al.*,2007).

The disease caused by this parasite (*haemonchus contortus*) is prevalent wherever sheep and goats are raised, but it exerts the greatest economic losses in temperate and tropical regions (Chaudary *et al.*, 2007). The disease has also found in the colder climates and recently been found as far north as the Arctic Circle (Durrani *et al.*, 2007).

Sheep are an important source of high quality food products for human (meat, milk) . Gastrointestinal nematodes are among the most destructive pathologies affecting ovine production, given the high prevalence in flocks and the serious consequences of the infection. In lambs, the acute form (the most frequent) of gastrointestinal nematode infection causes death. Chronic form leads to notable reduction in productivity in ruminants (Francisco *et al*, 2007).

The causative agent of *haemonchosis* in small ruminant is *haemonchus contortus*. This parasites is common blood feeders that cause anemia and reduce productivity and can lead to death in heavily infected animals. It has been estimated that each worm sucks about 0.05 ml of blood per day by ingestion or seepage from lesions (Qamar *et al.*, 2011).

Haemonchus contortus is the widely distributed in all geographic areas. Clinical signs of the infection include anemia, impaired digestion absorption syndromes and in many cases, the death of animals. The severity of the disease depends on a variety of factors, including the

number of helminthes infecting an animal (intensity of the infection). Isolate-related parasite virulence , host age, animal breed, and the nutritional and immunological status of the infected sheep . All these factors can be aggravated by local environmental conditions (Francisco *et al.*, 2007).

Ovine haemonchosis occurs in Three forms :per acute , acute, and chronic .The per acute form is less common and the infected lambs may die suddenly from severe hemorrhagic gastritis. Lambs and young sheep are commonly affected by the acute form of the disease , in which animals are found dead without showing overt clinical signs (Fayza *et al.*, 2003)

Ovine haemonchosis occurs mainly in Lambs and adult sheep could to be affected, The disease occurs in three Forms: per acute, acute and chronic (Blood and Radostitis, 1989).

Chronic haemonchosis was observed under field conditions in East Africa, it is usually associated with deteriorating pasture quality and low parasite burden and represents a major factor all over the world (Fayza *et al.*, 2003).

Several epidemiological studies on the GIN infection were carried out to depict the seasonal pattern of *haemonchosis* in different agro ecological areas of the world (Chaudary *et al.*,2007). The seasonal trend in *haemonchosis* is influenced by a number of abiotic and biotic factors that dictate the development and survival of pre-parasitic stages of *H.contortus* onto the herbage (Chaudary *et al.*,2007).

Justifications:

Sudan is a tropical country with an area of about 2 Million Square kilometer and sheep population 40 million Head. They are raised under different mange mental system in desert and semi desert area. Thus these worms represent a serious problem in sheep industry in the Sudan , this result in reduced yield of meat and milk, lowered the resistance to many other diseases. Many reports on the prevalence of *haemonchus contortus* infection in small ruminant, at different regions in Sudan may indicated that the pre acute could represent a major problem to sheep industry in the country (Fayza *et al.*,2003)

Moreover for many years, several methods were used to control this parasite. The use of anthelmintic is the most extensively use to control these nematodes (Getachew *et al.*, 2007).

However Khartoum is one of the states of the Sudan which has a large number of sheep population which is exposed to *Haemonchosis*, therefore determination of the prevalence of the disease in khartoum is very important in order to explore the size of the problem which helps prevent and control of the disease.

This study was carried out to obtain more information of the disease and to provide recommendation by fulfilling the following objectives:

Objectives :

1- To estimate the prevalence of *sheep haemonchosis* in Khartoum state.

2- To investigate potential risk factors associated with with sheep *haemonchosis* in Khartoum state..

CHAPTER ONE

LITERATURE REVIEW

Haemonchosis is a serious parasitic disease of domestic and wild animals in the tropics caused by the genus *Haemonchus* (Fabiya, 1987).

Haemonchus contortus is the species that most commonly infects sheep and goats and to a lesser extent bovine and cameline species (Fayza *et al.*, 2003).

Ovine haemonchosis :

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

1.1.2 Classification:

Kingdom: *Animalia*.

Phylum : *Nemathelminthes*.

Class: *Secernetea*.

Order: *Strongylida*.

Suborder: *Trichostrongylina*.

Superfamily: *Trichostrongyloidea*.

Family: *Haemonchidae*.

Subfamily: *Haemonchinae*.

Genus: *Haemonchus*.

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

The genus *Haemonchus* apparently originated in Africa, with an initial diversification in antelopes and subsequent colonization and development in other wild ruminants (Francisco *et al.*,2007). There were independent colonization , in domestic ruminants later, human migrations enabled the spread of *Haemonchus* to wild and domestic ruminants in other continents and in sheep as well . In addition , eleven more species have been described in the genus which are *H. semilis*, *H. longistipes*, *H. placei*, *H. bedfordi*, *H. mitchelli* ,*H. vegliali* ,*H. Iawrencei*, *H. Okrugeri*, *H. horaki* ,*H. dinnik*. Moreover, hybrids of *H. contortus* and *H. placei* have arisen. (Francisco *et al.*,2007).

1.1.3 Morphology:

An adult *haemonchus contortus* measures about 25 to 34 mm long, the male being shorter than the female which measure about 19 to 22mm, the morphological characteristics of *haemonchus contortus* are a mouth capsule with a single dorsal lancet and two prominent cervical papillae in the oesophageal area (Eseta, 2004). It has a tooth or lancet in its poorly developed oral cavity to perforate the gastric mucosa and suck blood the ingested blood gives the helminth a reddish coloration (Francisco *et al.*,2007). The male parasite is characterized by its copulatory bursa formed of two large lateral lobes and a small asymmetrically positioned dorsal lobe . Together with the two chitinous spicules , which are inserted in the female genital opening during copulation (figure.1), 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 a sharply pointed slender tail and

a vulva with or without anterior vulval flap (Eseta, 2004) (figure.2), 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. 3) . Eggs are typical of the Trichostrongyloidea Super family (Jones *et al*, 2010) . 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.4) .



Haemonchus - male Bursal lobes

(figure.1)



Haemonchus - female vulva area

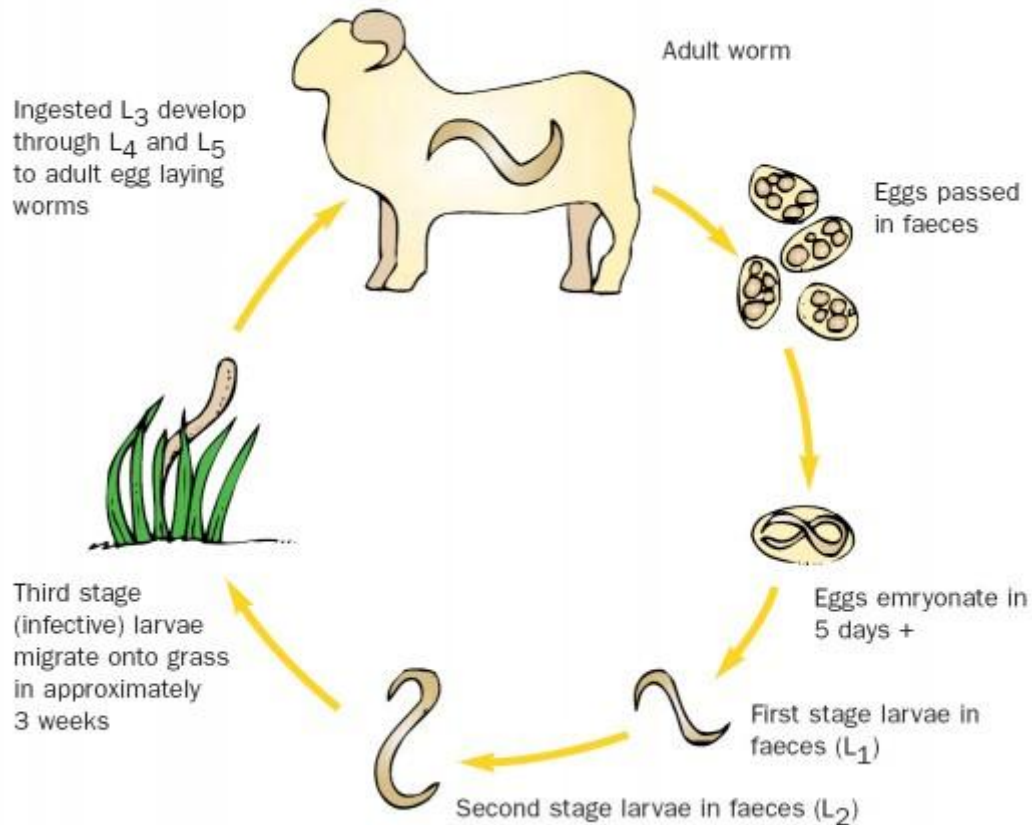
(figure.2)

1.1.4 : Life cycle of *Haemonchus contortus*:

The focus of the present thesis is on the nematodes residing in the gastrointestinal tract of small ruminants. These 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).

General nematode life cycle



(figure.5) general nematode life cycle.

The worms occur in the abomasum or fourth 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 (Brown, 2011). The life cycle of *H. contortus* consists of free-living stages on the pasture and parasitic stages within the host’s abomasum (Terefe, 2005) (Figure 6) .

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 trichostrongyle worms penetrate the epithelial layer of the mucus membrane (in the case of *Haemonchus* and *Trichostrongylus*) or enter the gastric glands (Teladorsagia).

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 undissociated carbonic acid in the gut. The now 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).

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 (referred to as the prepatent period) varies between parasite species, but generally 3 to 5 weeks (Sissay, 2007).

The *H. contortus* infective larvae can survive beyond 21 days in the soil and infest pasture grasses when the climatic conditions are favourable (Amaradasa *et al.*, 2010). Adult worms only survive for several months in the host (Paddock, 2011).

The free-living stages of *H. contortus* 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 very high 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).

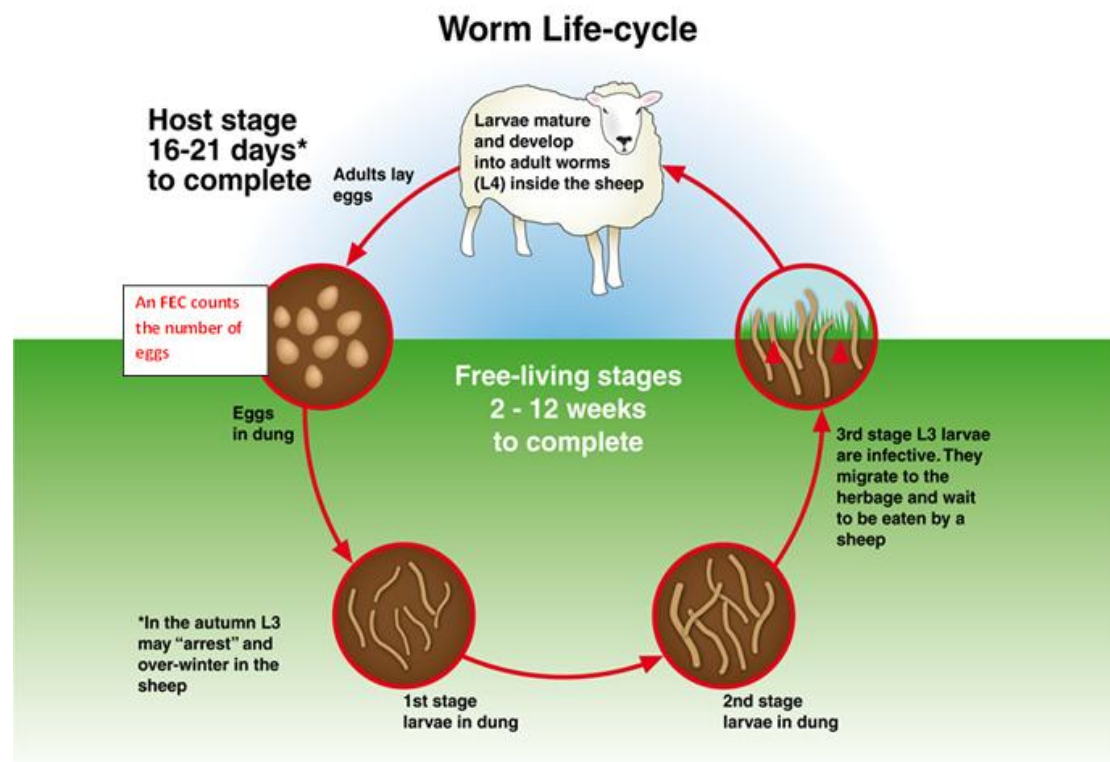
1.1.5 : Hypobiosis phenomenon:

An important phenomenon observed in the life cycle that has epidemiological implications is “arrested larval development” or “hypobiosis”.

Hypobiosis 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 (Soulsby,1982).

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, 2003).



General life cycle of gastrointestinal nematodes of small ruminants (figure.6)

1.2 Clinical manifestations and Signs:

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 to anaemia resulting from blood loss. With the exception of the L3, all other stages of development feed on blood. *Haemonchus contortus* is known to produce calcium and a clotting factor binding substance known as calreticulin , enabling the parasite to feed easily on host blood (Getachew *et al.*, 2007).

1.3 Diagnosis:

A) Direct smear method:

Pin point 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).

B) Modified Mc master technique:

The Mc master technique was use for counting the number of eggs per gram (egg/pg) in feces by suspending the faecal material in a saturated salt solution. Four grams of each faecal sample were weighted with the help of sensitive balance and place in a plastic beaker (250ml). The faecal pellets were mashed fully with the help of mortar. About 56ml

of water was added into the beaker and mixed well along with the faeces. The solution was homogenized for one minute with the help of Pasteur pipette. One ml of sugar solution was placed in the test tube with the help of pipette and added 1 ml of faecal solution to the test tube with the same pipette. The solution was mixed thoroughly. The faecal material passed through sieve to remove debris prior to pouring in Mc master chambers. The prepared samples was taken up with pipette and dispensed into both chambers of the Mc master counting slide (each slide comprising two chambers each of 10x10 mm; the space between object-glass and cover slip was 1.5 mm and each compartment contains of 0.15 ml). The number of the eggs (ova) of *haemonchus contortus* within the both grid of the chamber was counted, using microscope with magnification power of 10X. The number of eggs per gram of a faecal sample was obtained by the number (X) of eggs.

Eggs/gram (EPG) of faeces = total number of eggs count in the two chambers of Mc master slide multiply by 50 (Lynda *et al.*,2011).

C) Counting of worms:

Following slaughter and evisceration, the entire abomasa of sheep and goats slaughtered at abattoirs during the study period were collected and examined as described by Hansen and Perry (1990).

The abomasa were placed in a plastic container and transported to the laboratory. The entire washings from the abomasa were completely examined individually for worms. The *haemonchus contortus* present were identified and counted (Qamar, 2009).

D) Identification of larval stages (L3):

Faeces pools were carried out in each group for the faecal culture the method described by MAFF(1986). The identification of the third stage larvae (L3) was done using the key described by Gruner and Raynaud (1980); MAFF, (1986). These samples were cultured, the larvae recovered were harvested using after that Baermann's technique; The larvae were identified and counted using modified method from that described by Martin 1990 (Qamar, 2009).

E) 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 will float to the surface and may be removed by touching the surface with the end of a square – cut glass rod and transferred to a slide , alternatively the surface may be touched with a cover slip . if a 33% solution of zinc sulphate is used some eggs e.g those of fasciola hepatica , may be distorted and in addition , the higher the specific gravity of the flotation solution , the more debris is produced in the preparation the specific gravities of various common solution used in diagnosis are : saturated sodium chloride 1.200 , sugar (sucrose) 1.12-1.30 , zinc sulphate 33% 1.18 , magnesium sulphate 35% 1.28 , sodium nitrate 1.36 and potassium mercury iodide (111 g potassium iodide , 150 g mercury iodide , water 399 ml) 1.44 (soulsby 1982).

F) Serological and molecular diagnosis:

- Serological:

Detection of infection during prepatency, is of greater importance from the clinical point of view, therefore a simplified, field oriented Dot-ELISA has been developed for the detection of *haemonchus contortus* 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 (Lone *et al.*, 2012).

- Molecular diagnosis:

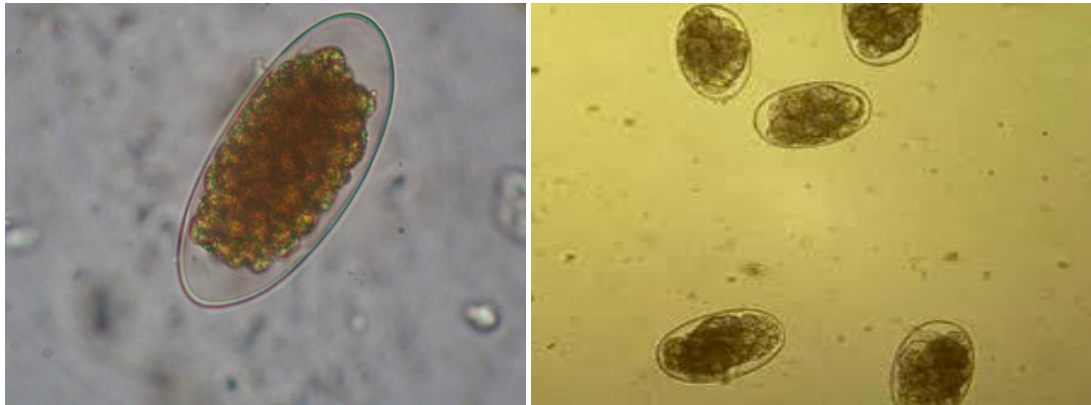
by using polymerase chain reaction specific primers and amplicon for each target cDNA, a primer SYBR green real-time PCR assay on a gene amplicon 5700 sequence detection system (applied biosystems) and based on known ovine gene sequences (β -actin, IFN- γ , TNF- α , IL-3, IL-4, IL-5, IL-10, IL-12p40). Oligonucleotides were designed to amplify a product with a size of 51 base pair, with a melting temperature (T_m) 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 μ g/ml, 24 hours in a 5% CO₂ and 37°C atmosphere) was then performed (Getachew *et al.*, 2007).

New diagnostic tool for monitoring parasites of sheep:

Dipstick assay for *haemonchus contortus* infection :

Commercial test-strips for detection of blood were found to provide a sensitive method for detecting the presence of *haemonchus contortus*

infections, in the fecal blood even before worms have matured into adults and commenced laying eggs. A provisional patent has been lodged and an extensive field trial. The test is performed on-farm using small fecal samples from a representative number of sheep. One limitation of the test is that it will not alert graziers to the presence of worms other than *haemonchus contortus*. Consequently, it will still be necessary to conduct conventional worm egg counts and larval differentiation tests until on-farm tests are developed for other worm species (Colditz *et al.*,2006).



Egg of *Haemonchus spp* (Left) and *Haemonchus contortus* egg where examined on day zero (Mostafa *et al.*,2013) (Right) (figure. 4).



blood in the gut of the parasite and the oviduct around the gut giving the characteristic barber's pole appearance (figure. 3)

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 (Tehrani *et al.*, 2012).

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 *haemonchus contortus* 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 occurrence 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:

i) 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 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 tegumental 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)

Include theimidazothiazoles (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 (Abbott *et al.*,2009).

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

Includes 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 (Abbott *et al.*,2009).

ii) Narrow spectrum anthelmintics:

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. The fasciolicides are discussed further in Section (Peregrine *et al.*,2006).

Praziquantel is a quinoline-pyrazine and is active against the tapeworm, *Moniezia expansa*. The drug acts on cell membrane permeability leading to damage to the parasite integument. Praziquantel is only available in combination with levamisole (Peregrine *et al.*,2006).

1.6.2 Some control strategy:

i) Vaccination:

The parasite gut provides a potential source of protective antigens. In fact, substantial protection can be induced against *haemonchus contortus* 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 *Haemonchus contortus*, 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 (Nayebzadeh *et al.*, 2008).

ii) 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).

iii) 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).

iv) Provision of “Safer” pasture:

The main methods of potentially achieving this are :-

- Grazing hay or silage regrowth.
- Cultivation and establishment of new pasture or forage crops for grazing with susceptible stock.
- Using areas previously grazed by a different ruminant species or a non-infective /immune stock class of the same ruminant species.

Hay or silage aftermath: paddocks are usually closed for 40-60 days before hay or silage is cut and removed, and then it is several more weeks before the regrowth is grazed. This time interval combined with the harvesting removes a large proportion of the larvae ; If the cutting height is above 5 cm, fewer larvae are likely to be removed than if the pasture is cut lower. Most of the larvae that remain on hay stubble should be killed by ultraviolet radiation and desiccation. Some contamination can remain,

especially if the areas were previously grazed by contaminating stock or if the spell is not long enough . Generally the area of such prepared pasture on most farms is too small to provide sufficient safe grazing for susceptible animals, and sooner or later they will have to graze contaminated pasture (Rattary, 2003).

New pasture and summer forage crops: These are generally considered to be free of internal parasite larvae Newly established pasture areas have not had any contamination for a long period of time and cultivation should have ensured that very few, if any, larvae survive. Generally the area of new pasture is limited. With specialist crops there is generally a long interval between the last grazing of pasture and the establishment and grazing of the crop. This interval and the cultivation should ensure few larvae are present. The physical structure of many fodder crops may preclude the migration of any larvae present into the grazing zone, although grass margins may remain a potential source of larvae. In many situations, such crops may be impractical (hill country) or not economic. In some situations where serious drench resistance has arisen, such as on goat units, taking non-forage crops such as potatoes for two to three years has cleaned the area up (Rattary, 2003).

Areas grazed by non-infective animals: pastures from which all infective sheep or goats have been excluded for at least 2-3 months but which have been grazed by cattle during that time can provide safe pasture for sheep or goats and vice versa. This is because they share very few of the same species of worm parasites and cross contamination of pasture by the alternate ruminant species is likely to have minimal infectivity for the principal species. This does not imply that absolutely no cross transmission can take place, but that a high proportion of ingested larvae do not establish in the heterologous host. Those that do,

often have a limited, if any, period of patency (egg production) as adults. One ruminant species can essentially clean up pasture contaminated by the other. Cattle are an appropriate alternate species to sheep. In the case of cattle, preparing pasture for lambs, once lambs have grazed and contaminated a paddock with worm eggs, subsequent grazing by cattle will help remove a proportion of any larvae that develop in the following ways: cattle act as vacuum cleaners. As they graze, they ingest larvae and those of sheep origin do not establish in cattle and hence die. (Likewise, sheep will help to remove some of the larvae that originate from cattle). Cattle grazing opens up the sward, exposing the larvae to desiccation and ultraviolet radiation. In addition, cattle grazing can increase the white clover content of swards. This can reduce the production losses due to parasitism as well as boosting lamb performance. Because of the extended interval before sheep return to the paddock, in some seasons there will also be considerable reduction in larval contamination through natural larval mortality (Rattary, 2003).

1.7 Epidemiology:

1.7.1 Geographical distribution:

Haemonchus contortus 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 18°C and approximately 50 mm rainfall. Thus it has been generally recognized that *haemonchus contortus* 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 (Chandrawathani *et al.* , 2005).

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 *haemonchus contortus* displayed by this study was 32% (Fayza *et al.*, 2003).

In a study a total of 498 fecal samples and 45 gastrointestinal tracts of sheep from central Kordofan were examined for gastrointestinal helminths. Mixed helminth infections were found to be common in 91.1% of gastrointestinal tracts examined. Nematode infections were the commonest, reaching 82.2% of the examined animals. *Haemonchus contortus* and *Trichostrongylus colubriformis* were, having the highest prevalence 68.9% and 60%, respectively. Other identified nematode species were *Cooperia pectinata*, *Oesophagostomum columbianum*, *Strongyloides papillosus*, *Trichuris globulosa* and *Skrjabinema ovis* with a frequency of 35.1%, 59.2%, 62.2%, 27% and 8.1, respectively. *Cestodes* were recovered in 57.8% of the gastrointestinal tracts. The identified species were *Stilesia globipunctata*, *Avitellina centripunctata*, *Moniezia expansa* and *Moniezia benedeni*. The most prevalent cestode species were *S. globipunctata* and *A. centripunctata* with a frequency of 37.8% for each species. There was a seasonal effect on nematode infection in sheep as judged by egg output and worm burden. Both parameters showed their highest levels during the rainy season (Ghada *et al.*, 2011).

In a study a gastrointestinal tracts of 79 sheep and 161 goat were obtained from abattoir of Tulus locality in south Darfur state in western Sudan from march 2006 to February 2007 and examined for the presence

of gastrointestinal parasites. Seven nematodes species (99.8%) and *monezia expansa* (0.2%) in sheep beside eight nematodes species (99.9%) and *monezia expansa* (0.1%) in goat were Identified. In sheep the nematodes were in order of prevalence: *Haemonchus contortus*(53.4%),*Strongyloides papillosus*(26.2%) *Trichostrongyloides colabrifomis*(14.7%), *Cooperia pectinata*(3.1%), *Oesophagastmum columbianum*(2.2%), *Skrjabinema ovis*(0.3%) and *Trichuris globulosa*(0.1%) while in goat were: *Strongyloides papillosus*(26.5%), *Haemonchus contortus*(26%), *Trichostrongylus colubrifomis*(24.4%), *Skrjabinema globulosa*(0.6%) and *Cooperia pectinata*(0.1%).The intensity of the parasite infection was light to moderate. The mean worm burden was 497.3 and 472.4 for sheep and goat respectively. The total worm burden was shown has association with season and sex in goat but not in sheep while no association was observed between total worm burden and age of the animals in both sheep and goats. The effect of climatic factors on worm burden revealed a significant positive correlation with rainfall and relative humidity but not with temperature (Almalaik *et al.*, 2008).

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 Sheikhpura (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 9

months of age than in above 9 months of age(33.08%). Animals of either sex were equally affected (Qamar *et al.*, 2009).

A cross sectional study was conducted from November 2011 to March 2012 to determine the prevalence of gastrointestinal (GI) helminth infections and associated risk factors in sheep and goats in and around Mekelle town, northern Ethiopia. A total of 390 small ruminant's faecal samples (240 sheep and 150 goats) were collected and examined using standard parasitological procedures. The study revealed that the overall prevalence of helminthiasis was 56.25% and 35.33% in sheep and goats, respectively. A statistically significant difference ($p < 0.05$) was found in prevalence between sheep and goats. *Strongyles* were the most prevalent parasites encountered in the Study area followed by *Trichuris spp.* Sex and body condition of the animals were shown to have association with prevalence and significant difference ($p < 0.05$) was also found. A statistical significant difference was not observed ($p > 0.05$) in prevalence with age of animals. potential risk factors for the occurrence of the disease should be considered in designing strategic anthelmintic treatment (Negasi *et al.*, 2012) .

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 anaemia during dry Seasons (Attindehou *et al.*, 2012).

A study was carried out to determine the prevalence, species composition and worm burden of abomasal nematodes of small ruminants of Ogaden region slaughtered at Elfora export abattoir. A total of 196 abomasums of animals (114 sheep and 82 goats) were examined according to standard procedures. An overall prevalence rate of 91.2% and 82.9% *haemonchus* Species was recorded in sheep and goats, respectively. Likewise, an overall prevalence of 37.7% and 40.2% *Trichostrongylus axei* was recorded in sheep and goats, respectively. Statistically significant ($p < 0.05$) difference in prevalence and average worm burden was noted between months of study for both abomasal nematodes. Majority of sheep and goats harboring adult abomasal nematodes were with light to moderate degree of infection where only small proportions were with heavy degree of infection. Adult male *haemonchus* worms collected from sheep were identified as 95.1% *haemonchus contortus* 3.4% *haemonchus placei* and 1.2% *haemonchus longistipes*. Similarly, male *haemonchus* recovered from goats were identified as 96.5% *haemonchus contortus*, 3.0% *haemonchus placei* And 0.5% *haemonchus longistipes*. The study revealed the coexistence and sympatry of communities of two or three *haemonchus* species in a single small ruminant host, suggesting occurrence of *haemonchus* species

circulation among heterologous hosts sharing the same pastures that should be considered in the control strategy of the parasite (Kumsa *et al.*, 2006).

From August 2004 to December 2005, a total of 338 fecal samples were collected (86 from sheep 252 from goats) to determine the prevalence of various endo parasites in and around twin cities of Rawalpindi and Islamabad. of the total samples examined, 65.7% were found positive for endo parasites. The prevalence of gastrointestinal parasites tended to be higher ($p = 0.059$) in sheep 62 (72%) than in goats 160 (63.7%). The endo parasites identified in sheep included *haemonchus* (80.64%), *Coccidia* (51.61%), *Trichuris* (32.25%), *Nematodirus* (29.03%) and *Fasciola* (4.38%) while only *haemonchus* (75%), *Trichuris* (62.5), and *Coccidia* (57.5%) were recovered from the fecal samples of goat (Asif *et al.*, 2008).

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 *haemonchus contortus* 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 study was carried out to determine the prevalence of abomasal nematodes of sheep and goats slaughtered in Behshahr town from January 2012 through June 2012 with special emphasis given to *Haemonchus* species. During the study period 200 abomasa of sheep and 200 abomasa of goats were examined according to standard procedures. Three genera of nematodes were identified in both sheep and goats abomasa with overall prevalence of 88 % (n = 200). And 79.5 % (n = 200) respectively. The specific prevalence rates observed were 69.3% for *Haemonchus* spp., 37.8% for *Parabronema skerjabini*, and 13.7% for *Teladorsagia* spp. in sheep and 69.8 % for *Haemonchus* spp., 31.3 % for *Parabronema Skerjabini* and 11.8% for *Teladorsagia* spp. in goats. Generally a high infection rate with abomasal nematodes was observed in both sheep and goats of the study area (Garedani *et al.*, 2013).

From August 2004 to May 2008, a total of 400 submitted faecal samples comprising of 90 samples from sheep and 310 from goats of

Rawalpindi and Islamabad were analyzed to confirm the presence of gastrointestinal parasitic infection. 254 (63.50%) samples were found positive for endo parasites. Among the samples from sheep 48 (53.33%) and 206 (66.45%) from goats were detected positive for gastrointestinal parasites. *Trichuris*, *Haemonchus*, *Coccidia*, *Nematodirus* and *Fasciola* were found with prevalence of 40.00, 28.88, 27.77, 11.11 and 4.44 per cent respectively in sheep. In case of goat the incidence of *Haemonchus*, *Coccidia*, *Trichuris*, *Nematodirus*, *Trichostrongylus*, *strongyloides* and *Fasciola* were 64.19, 43.87, 35.48, 13.00, 4.51, 3.22 and 0.66 % respectively (Gadahi *et al.*, 2009).

A survey on the prevalence and intensity of infection with gastrointestinal helminths of Dorper sheep in relation to age and weather factors was carried out on a ranch in Kajiado district, a semi-arid area of Kenya. Faecal samples from lambs (3 months to 1 year), yearlings (1–2 years) and adult breeding ewes (2–4 years) were examined for helminth egg output and helminth genus composition at 3-week intervals. The results indicated that the prevalence of *Strongyle* and tapeworms infections were highest for lambs, followed by the adult breeding ewes and then for the yearlings. In all age groups the proportions of infected animals were higher during the wet season than in the dry season for both nematodes and tapeworms. The mean *Strongyle* egg counts were higher during the dry season for lambs, but were higher during the wet season for the other age groups. Mixed *strongyle* infections were detected, with *Trichostrongylus* (55%), *Haemonchus* (28 %), *Cooperia* (10.5 %) and *Oesophagostomum* (6.5%) being the most frequently encountered genera throughout the study period. The trends in *Strongyle* faecal egg counts indicated the occurrence of Hypobiosis, with resumption of development towards the end of the dry season and at the onset of the

short rains in October and November. Self-cure was also observed in September and November in all age groups, although less frequently in lactating ewes. The prevalence and intensities of infection with gastrointestinal helminths in this area appeared to be influenced by the age of the host and weather factors (Ng'an'ga *et al.*, 2004).

Some epidemiological aspects of *Trichostrongylid nematodes* infecting gastrointestinal tract of Sheep were Studied in a part of agro-ecological zone of Pakistan. Six different species of *Trichostrongylid nematodes* viz., *Haemonchus (H.) contortus*, *Trichostrongylus (T.) axei*, *T. colubriformis*, *Ostertagia (O.) trifurcata*, *O. circumcincta* and *Cooperia (C.) curticei* were identified from 960 gastrointestinal tracts of sheep Slaughtered at local abattoir of Faisalabad. *Haemonchus contortus* was the highest in prevalence (61.5%) followed by *Trichostrongylus species* (46.1%), *Ostertagia species* (33.0%) and *C.curticei* (18.5%). A majority (94.6%) of the infected sheep harbored more than one species of nematode parasites, having minimum two and maximum three *Trichostrongylid nematode* species in each host. The highest prevalence was recorded in the months of July, August and September. A trend of higher prevalence was recorded in young animals and in females. An association of prevalence, worm burden, arrested larvae, availability of nematode larvae on pasture and periparturient period was observed. The results warranted development of a strategic worm control program in the area of study, which at present is not in practice anywhere in Pakistan (Lateef *et al.*, 2005).

Another study was conducted to determine the prevalence of *haemonchus contortus* in slaughtered sheep at Urmia abattoir located in the North west of Iran. A total of 2421 animals were slaughtered and

examined from July 2010 to July 2011 in Urmia abattoir. In case of sheep, 225 out of 2421 were positive and prevalence of *haemonchus contortus* infestation was 9.3%. Sex wise prevalence of *haemonchus contortus* in sheep was 33.08% (76/229) in male and 66.22% (149/225) in female. The females indicated significantly ($P < 0.05$) higher prevalence (66.22%) as compared to males (33.08%). The highest prevalence was recorded in the spring (April) and the lowest was in summer (July), respectively. On microscopic examination, infiltration of mononuclear cells and eosinophils in gastric glands, periglandular hyperemia and hemorrhage, mucous gland hyperplasia, connective tissue proliferation and necrosis was observed. Also, in mixed abomasal infection with *Haemonchus* and *Ostertagia* species, mucosal hyperplasia and increased mucous glands and sometimes cystic glands were seen. Statistical analysis using SPSS software, and chi-square test, demonstrated a non-significant difference between ages and abomasal pH values of infected and Healthy Sheep ($p < 0.05$). But the difference between sexes, seasons and abomasal lesions was significant ($p > 0.05$) (Tehrani *et al.*, 2012).

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 ($X^2 = 1.607$, $p > 0.05$). 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 ($X^2=1.727$, $p>0.05$) 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 *et al.*, 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 town 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 Study area:

The study was carried out in Khartoum State which has desert and semi-desert climate. The state receives little infrequent rain with an average less than 300mm per year, and is characterized by three distinct seasons; cold-dry from November to February, hot-dry from March to June, and hot-wet season from July to October; and wide diurnal and annual temperature variations. The highest mean maxima occur during March-May, ranging between 35-43°C and the lowest mean minima occur during December-February ranging between 15-28°C (Adel, and Omer 1999).

2.2 Study design:

A cross sectional survey was conducted to determine the prevalence of sheep *haemonchosis* and to investigate potential risk factors associated with the disease. Using multistage random sampling the study areas were selected; at first level, Khartoum city was divided into three towns Khartoum, Bahri and Omdurman. In the second, level, into seven Locality Omdurman , Um bada , karary , Khartoum , Jabal al Awliya , bahri , and East Nile were selected all localities . In the third level, the study areas were selected according to the proportion of sheep within the locality. The distribution of the sample in the localities was as following : 37 animal from Omdurman, 10 animal

from Um bada , 11 from karary , 30 animal from Khartoum locality, 25 from Jabal al Awliya, 40 from bahri and 17 animal from East Nile . In each locality different areas were selected randomly.

2.3 Sample size:

The sample size was calculated according to Martin *et al.*,(1987). formula:

$$n = \frac{4 \times P \times Q}{L^2}$$

Where:

n≡ Required Sample Size

P≡ Expected prevalence = 12.1

Q ≡ 1- P = 1-12.1

L≡ Allowable error =(0.05)

The previous prevalence selected equals 12.1% according to (Mohammed, 2014) in a study conducted in a same area in Khartoum state . The confidence level was chosen as 95% and the allowable error equal 5%

$$n = \frac{4 \times 12.1 \times (1 - 12.1)}{0.0025} = 170$$

The small sample size calculated (170).

2.4 Sample collection and laboratory diagnosis :

2.4.1 Faecal collection:

Faecal samples were collected directly from the rectum weekly in plastic container and transported to Sudan University laboratories for diagnosis.

2.4.2 laboratory diagnosis :

A) Direct smear method:

Pin point amount of faeces 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 the slide was examined under microscope for the presence of *haemonchus contortus* eggs (Qamar, 2009).

B) Centrifugal flotation :

A sample of feces Weighed or measured by using a pre calibrated teaspoon approximately 3g of faeces were put into container, mixed with water (about 30-50 ml) and strained through a sieve (1 mm mesh) to remove coarse faecal material . the mixture was sedimented for 10 minutes on the bench , or by light ceterifugation until the supernatant was clear.

Then the sediment mixed with a saturated solution of (NaCl) in a centrifuge tube (15-50 ml volume) and centrifuged for three minutes at 500 g . the eggs will float 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

D) Faecal samples culture and Baermann technique:

Faecal culture:

The faecal samples was braked up in a container with spatula. The charcoal was added to the wet faeces and the water was added to the dry faeces. All the faecal sample was incubated at 27⁰c for 10-14 days.

Baermann technique:

Funnel supported on a single stand with a short tube to the stem. The tube was closed with the clamp. Double layer cheesecloth was placed on a disposable paper towel. In each sample 10 grams of faecal material was placed in the center of the cheesecloth the cheesecloth pouch was closed by using a length of string and a short stick was pushed under the rubber band so that the pouch can be suspended. The pouch was placed in the funnel, the excess cheesecloth was trimmed off , the funnel was filled with lukewarm water until the faecal material was covered then the apparatus was leaved for 12 hours to take the larvae leave the faecal material and collected by the gravitation in the stem of the funnel. A few milliliters of the fluid in the stem was drawn off into the test tube by releasing the clamp slowly. This fluid was drawn into centrifuge tube and spun at 1000 r.p.m for 2 minutes. The sedimented sample was checked in a petri dish for the presence of the larvae (Lynda *et al.*,2011).

Identification of *haemonchus* Larvae:

Was taken by Pasteur pipette; small droplet of the sedimented fluid recovered by Baermann technique was

transferred to a microscope slide and cover slip was gently placed over the drop. Drop of iodine was added to fix the larvae; the nematode larvae stain dark brown (Lynda *et al.*,2011).

Characters used for Identification:

The Identification of *Haemonchus* third stage larvae based on the following morphological features:

- Have a kinked sheath
- Have a rounded head.
- Have a pointed tail
- Medium size of tail sheath (Lynda *et al.*,2011).



Haemonchus contortus larvae (L3) after faecal samples cultured before starting the study (Mostafa *et al.*,2013) (figure. 7)

2.5 Questionnaire survey:

Owners and/or managers of all farms involved in the study were asked to provide information about potential risk factors suspected to be associated with *haemonchosis*. The questionnaire included information about breed, age, sex, body condition score, fecal consistency as individual risk factors. Also, housing, source of water, use treatments, presence of other animals, owner knowledge were included as Management risk Factors. Also, vegetation , type of soil , localities as climatic risk factors. These potential risk factors were divided into categories (Thrusfield , 2007).

2.6: Data analysis:

The data collected were entered into computer on micro soft excel spreadsheet. Statistical analysis was performed using ‘statistical package for the social sciences’ (SPSS), version 16. Frequency tables for the distribution of the potential risk factors and cross tabulation of sheep *haemonchosis* according to the potential risk factors was conducted.

Associations between the outcome variable (status of *haemonchosis*) and the potential risk factors were first screened in a univariate analysis using chi-square test. Potential risk factors with P-value ≤ 0.25 (two tailed; $\alpha = 0.25$) were considered significant in the χ^2 test. A multivariate model for the outcome variable was constructed using logistic-regression analysis. Risk factors with a p-value equal or less than 0.05 were considered significantly associated with sheep *haemonchosis*.

Chapter Three

Results

3.1.1 Results:

A total of 170 sheep were tested by Direct smear method and flotation test and larval identification by faecal culture using Baerman technique. Eleven animals were found positive (6.5%) and 159 animals were found negative (93.5%) for sheep *haemonchosis* (diagnosed by flotation) (Table: 1) . Therefore the overall prevalence of sheep *haemonchosis* in Khartoum state was 6.5% , and 8.2% by larval identification (Baerman technique) .

Table 1 : Frequency table for the prevalence of *haemonchus contortus* in 170 sheep from Khartoum state diagnosed by Centrifugal flotation test .

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid				
-ve	159	93.5	93.5	93.5
+ve	11	6.5	6.5	100
Total	170	100	100	

3.1.2 Localities :

The results showed that the overall prevalence of *haemonchosis* out of 170 examined sheep in Khartoum state was 6.5% by flotation test , The highest rate of infection was in karary (18.2%), and East Nile (17.6%) and bahri (12.5%) and the rate in

Omdurman was (2.7%) . Whereas the lowest rate of infection was in Khartoum (0%) and Jabal al Awliya (0%) and um bada (0%) (Table 2) and (Table 3).

The Chi square test, showed that there was significant association between *haemonchus contortus* infection and locality (p-value=0.032) (Table 4).

3.1.3 Sex of animal :

The results of study showed the distribution of 170 sheep examined for *haemonchosis* by sex. Total number of female examined was 80 animals. Among these, 5 animals were found infected. The rate of infection was 6.3%. Total number of males examined was 90 . Among these, 6 animals were found infected. The rate of infection was 6.7% (Table 2) and (Table 3).

The Chi-square test, showed that there was no significant association between *haemonchus contortus* infection and sex of animal (p-value =0.912) (Table 4).

3.1.4 Age of animal :

The results of study showed the distribution of 170 sheep examined for *haemonchosis* by age. Total number less than or equal 1 year of age was 45 animals. Among these, 3 animals were found infected. The rate of infection was 6.7 %. Total number of animals more than 1 years was 125 animals. Among these, 8 animals were found infected. The rate of infection was 6.4% (Table 2) and (Table 3).

In the Chi-square test, the result showed that there was no significant association between *haemonchus contortus* infection and age of animal (p-value =0.950) (Table 4).

3.1.5 Body condition of animal:

The body condition of animals and the presence of *haemonchus contortus* was been investigated. 160 animals were found in good condition. among these 10 animals were found infected. The rate of infection was 6.3 %, and 10 animal in poor condition among these one animal was found infected and the rate of infection was 10%.(Table 2) and (Table 3).

The Chi square test showed that there was no significant association between *haemonchus contortus* infection and body condition (p-value=0.640) (Table 4).

3.1.6 Breed of animal:

The results of study showed distribution of *haemonchus contortus* infection in Khartoum state by breed. The number of Hamari breed examined was 52 animal, Among these, 2 animals were found infected. The rate of infection was 3.8%. The number of Kabashi breed examined was 106 animals. Among these 6 animals were found infected. The rate of infection was 5.7%. While number of dubasy breed examined was 12 animals. Among these 3 animals were found infected. The rate of infection was 25% (Table 2) and (Table 3).

The Chi square test showed there was significant association between *haemonchus contortus* infection and breed (p-value=0.023) (Table 4).

3.1.7 Housing:

170 sheep of various housing types were examined in this study, and the presence of *haemonchus contortus* was investigated. 155 of sheep raised indoor were examined. Among these, 8 animal were found infected. The rate of infection was 5.2%. While 15 of sheep raised outdoor were examined. Among these, 3 animal were found infected. The rate of infection was 20% (Table 2) and (Table 3) .

The Chi square test showed that there was significant association between *haemonchus contortus* infection and housing (p-value =0.26) (Table 4).

3.1.8 Source of water :

The study results showed the distribution of *haemonchus contortus* infection in Khartoum state by source of water. The number of sheep examined which drink from tape water was 118 animals. Among these, 7 were found infected. The rate of infection was 5.9%. Whereas the number of sheep which drink from well was 37 animals. Among these, one animals was found infected. The rate of infection was 2.7% . and the number of sheep which drink from canal was 15 animals. Among these, 3 animals were found infected. The rate of infection was 20% (Table 2) and (Table 3).

The Chi square test showed that there was significant association between the *haemonchus contortus* infection and source of water (p-value =0.065) (Table 4)

3.1.9 Use of anthelmintic:

170 sheep categorized according to using of anthelmintic, and the presence of *haemonchus contortus* infection was investigated. Total animals number in the category of anthelmintic used was 165 animals. Among these, 9 animals were found infected. The rate of infection was 5.5% . While 5 animals in the category of anthelmintic not used. Among these; 2 animal were found infected. The rate of infection was 40% (Table 2) and (Table 3).

The Chi square test showed that there was significant association between *haemonchus contortus* infection and using of anthelmintics (p-value= 0.002) (Table 4)

3.1.10 owner knowledge:

170 sheep categorized according to owner knowledge , and the presence of *haemonchus contortus* infection was investigated. 116 sheep in the category of owner who know about the disease . Among these, 3 animals were found infected. The rate of infection was 2.6 % . While 54 animals in the category of owner who didn't know about the disease . Among these; 8 animal were found infected. The rate of infection was 14.8% (Table 2) and (Table 3).

The Chi square test showed that there was significant association between *haemonchus contortus* infection and owner knowledge (p-value= 0.003) (Table 4)

3.1.11 Other animals :

The study results showed the distribution of *haemonchus contortus* infection in Khartoum by presence of other animals

species with the sheep in the same farm. Total numbers of sheep which raised alone were 127 animals. Among these, 8 animals were found infected. The rate of infection was 6.3%. sheep raised in the presence of other animals species were 43 animals. Among these, 3 animals were found infected. The rate of infection was 7% (Table 2) and (Table 3).

The Chi square test showed that there was no significant association between the *haemonchus contortus* infection and present of other animals (p-value= 0.876) (Table 4)

3.1.12 Faecal consistency:

The results of study showed the distribution of *haemonchus contortus* infection in Khartoum by fecal consistency. Animals with normal feces were 164 animals. 11 animals out of them were found infected. The rate of infection was 6.7%. Animals with soft feces was 6 animals. Among them, no animals were found infected. The rate of infection was 0%.(Table 2) and (Table 3).

The Chi square test showed that there was no significant association between *haemonchus contortus* infection and fecal consistency (p-value= 0.512) (Table 4)

3.1.13 Vegetation :

The study results showed the distribution of *haemonchus contortus* infection in Khartoum state by vegetation condition. The number of sheep examined in present vegetation areas was 160 , Among these, 9 animal were found infected. The rate of infection was 5.6%. While the number of sheep in absent

vegetative areas was 10 , Among these, 2 animal were found infected. The rate of infection was 20% (Table 2) and (Table 3).

The Chi square test showed that there was significant association between *haemonchus contortus* infection and vegetation area (p-value = 0.073) (Table 4).

3.1.14 Types of soil:

170 sheep raised on various types of soil were examined for *haemonchus contortus* infection. Total number of sheep examined which raised in mud soil was 68 animals. Among these, 10 animals were found infected. The rate of infection was 14.7%. While 102 of animals which examined raised in sand soil . Among these, one animal were found infected. The rate of infection was 0.98% (Table 2) and (Table 3).

The Chi square test showed that there was highly significant association between *haemnochus contortus* infection and type of soil (p-value =0.000) (Table 4)

3.2 Results of multivariate analysis:

Eight potential risk factors were found to be significantly (P-value \leq 0.25) associated with sheep *haemonchosis* in the univariate analysis (Table 4) entered to logistic regression; final model, out of these eight risk factors, no any factors were significantly (P-value \leq 0.05) associated with sheep *haemonchosis* in the multivariate analysis (table 5) .

Table 2 : Summary frequency for the distribution of 170 sheep examined for *haemonchosis* by Centrifugal flotation test according to potential risk factors investigated :

Risk factor	Frequency	Relative frequency (%)	Cumulative frequency (%)
Sex :			
Female	80	47.1	47.1
Male	90	52.9	100
Age:			
≤year	45	26.5	26.5
>year	125	73.5	100
Body condition:			
Good	160	94.1	94.1
Poor	10	5.9	100
Breed:			
Kabashi	106	62.4	62.4
Dubasy	12	7.1	69.5
Hamari	52	30.5	100
Housing:			
Indoor	155	91.2	91.2
Outdoor	15	8.8	8.8
Source of water:			
Tape	188	69.4	69.4
Canal	15	8.8	78.2
well	37	21.8	100
Use of anthelmintics:			
Yes	156	97.1	97.1
No	5	2.9	100
Owner knowledge :			
Yes	116	68.2	68.2
No	54	31.8	100
Other animal:			
Yes	43	25.3	25.3
No	127	74.7	100
Faecal consistency:			
Normal	164	96.5	69.5
Soft	6	3.5	100
Vegetation :			
absent	160	94.1	94.1
present	10	5.9	100
Type of soil:			
Sand	102	60	60
mud	68	40	100
Locality:			
Bahri	40	23.5	23.5
East Nile	17	10.0	33.5
Omdurman	37	21.8	55.3
Um bada	10	5.9	61.2
Karary	11	6.5	67.6
Khartoum	30	17.6	85.3
Jabal al Awliya	25	14.7	100

Table 3 : Summary cross-tabulation of *haemonchosis* in 170 sheep examined by Centrifugal flotation test according to potential risk factors investigated :

Risk factors	Animals tested	Animals affected	Affected %
Sex :			
Female	80	5	6.3
Male	90	6	6.7
Age:			
≤year	45	3	6.7
>year	125	8	6.4
Body condition:			
Good	160	10	6.3
Poor	10	1	10
Breed:			
Kabashi	106	6	5.7
Dubasy	12	3	25
Hamari	52	2	3.8
Housing:			
Indoor	155	8	5.2
Outdoor	15	3	20
Source of water:			
Tape	118	7	5.9
Canal	15	3	20
well	37	1	2.7
Use of anthelmintics:			
Yes	165	9	5.5
No	5	2	40
Owner knowledge :			
Yes	116	3	2.6
No	54	8	14.8
Other animal:			
Yes	43	3	7
No	127	8	6.3
Faecal consistency:			
Normal	164	11	6.7
Soft	6	0	0

Table 3 Continued:

Vegetation : absent present	160 10	9 2	5.6 20
Type of soil: Sand Mud	102 68	1 10	1 14.7
Locality: Bahri East Nile Omdurman Um bada Karary Khartoum Jabal al Awliya	40 17 37 10 11 30 25	5 3 1 0 2 0 0	12.5 17.6 2.7 0 18.2 0 0

Table 4 : Summary of univariate analysis for risk factors associated with sheep haemonchosis in Khartoum State, Sudan (march to May 2015 ; n=170) using the Chi-square test.

Risk factors	No. inspected	No. affected (%)	Df	X²	p- value
Sex : Female Male	80 90	5 (6.3) 6 (6.7)	1	.12	.912
Age: ≤year >year	45 125	3 (6.7) 8 (6.4)	1	.004	.950
Body condition: Good Poor	160 10	10 (6.3) 1 (10)	1	.219	.640
Breed: Kabashi Dubasy Hamari	106 12 52	6 (5.6) 3 (25) 2 (3.8)	2	7.51	.023*

Table 4 Continued:

Housing: Indoor Outdoor	155 15	8 (5.2) 3 (20)	1	4.971	.026*
Source of water: Tape Canal well	118 15 37	7 (5.9) 3 (20) 1 (2.7)	2	5.461	.065*
Use of anthelmintics: Yes No	165 5	9 (5.5) 2 (40)	1	9.570	.002*
Owner knowledge : Yes No	116 54	3 (2.5) 8 (14.8)	2	9.105	.003*
Other animal: Yes No	43 127	3 (7) 8 (6.3)	1	.024	.876
Faecal consistency: Normal Soft	164 6	11 (6.7) 0 (0)	1	.430	.512
Vegetation : absent present	160 10	9 (5.6) 2 (20)	1	3.21	.073*
Type of soil: Sand Mud	102 68	1 (1) 10 (14.7)	1	12.70	0.000*
Locality: Bahri East Nile Omdurman Um bada Karary Khartoum Jabal al Awliya	40 17 37 10 11 30 25	5 (12.5) 3 (17.6) 1 (2.7) 0 (0) 2 (18.1) 0 (0) 0 (0)	6	13.76	.032*

*means significant value .p- value ≤ 0.25

Multivariate analysis using Logistic Regression models:

Risk factors that were significant (p-value ≤ 0.25) in the univariate model were re-entered in logistic regression in the final multivariate models. A variables with (p- value ≤ 0.05) was considered statistically significant

Table 5 :

Final logistic regression model of Sheep haemonchosis in Khartoum State, Sudan (March, to May, 2015 ;n=170)

Risk factors	Animals affected (%)	Exp(B)	95% Confidence Interval for Exp.(B)		p- value
			Lower	Upper	
Breed:					
Dubasy	2 (3.8)	Ref			
Hamari	6 (5.6)	1.741	.312	9.722	.528
Kabashi	3 (25)	8.23	.251	2.700	.748
Housing:					
Indoor	8 (5.2)	Ref			
Outdoor	3 (20)	1.448	.0107	19.653	.781
Source of water:					
well	1 (2.7)	Ref			
Tape	7 (5.9)	.718	.182	2.841	.637
Canal	3 (20)	9.000	.854	94.899	
Use of anthelmintics:					
Yes	9 (5.5)	Ref			
No	2 (40)	3.409	.308	37.766	.318
Owner knowledge :					
Yes	3 (2.5)	Ref			
No	8 (14.8)	1.389	.352	5.489	.639
Vegetation :					
absent	9 (5.6)	Ref			
present	2 (20)	1.363	.002	972.532	.924
Type of soil:					
Sand	1 (1)	Ref			
Mud	10 (14.7)	1.351	.226	8.057	.741
Locality:					
Jabal al Awliya	0 (0)	Ref			
Khartoum	0 (0)	.740	.124	4.416	.741
Um bada	0 (0)	.381	.000	297.843	.777
Omdurman	1 (2.7)	1.001	.103	9.698	.999
Bahri	5 (12.5)	1.399	.172	11.356	.753
East Nile	3 (17.6)	3.672	.004	2051.205	.705
Karary	2 (18.1)	1.026	.199	5.284	.976

* means significant value .p- value ≤ 0.05

Chapter Four

Discussion

Sudan with its estimated 40 million head of sheep, is one of the largest sheep breeding countries in Africa. Nevertheless little attention has been directed towards ovine *haemonchosis* in this country (Fayza *et al.*, 2003).

The occurrence of *haemonchosis* in an area is influenced by a multi-factorial system, which comprises hosts, parasite and environmental effects. *haemonchus* is common blood feeders that cause anemia and reduced productivity and can lead to death in heavily infected animals. It has been estimated that each worm sucks about 0.05 ml of blood per day by ingestion or seepage from lesions (Qamar *et al.*, 2009).

The results of this study indicated that *haemonchus contortus* is wide spread in sheep of Khartoum state, Sudan. Therefore the overall prevalence of ovine *haemonchosis* in Khartoum state, Sudan was 6.5%. This prevalence is lower than the prevalence reported in Omdurman slaughterhouse which was 32% (Fayza *et al.*, 2003) , and also lower than the prevalence reported in abattoir of Tulus locality in south Darfur state which was 53.4% (Almalaik *et al.*,2008) . while Gagood *et al.*,(1968) reported 80% it's a much higher rate prevalence of *H.contortus* among sheep examined in Omdurman slaughter house , This difference between the latter authors' findings and ours might have been due to seasonal variation .

The prevalence of sheep *haemonchosis* in our study is much lower than the prevalence in other studies in different countries which was 56.25 % in Ethiopia (Negasi *et al.*, 2012), 72.5 % also in Ethiopia (Sissay, 2007), 77.7 % in Pakistan (Tasawar *et al.*,2010), 78.1 % in Ethiopia (Abunna *et al.*, 2009), and 80.64 % in Pakistan (Asif *et al.*,2008).

Also the prevalence of sheep *haemonchosis* in this study is lower than the prevalence in the other studies in different countries which was 47.67% in Ethiopia (Dagnachew *et al.*, 2011), 55.56 % in Benin (Attindehou *et al.*,2012), 37.18 % in Pakistan (Raza *et al.*, 2009), and 35.44% also in Pakistan (Qamer *et al.*, 2009) and in Iran 9.3% (Tehrani *et al.*, 2012).

The differences in prevalence reported by these studies could be accounted on the basis of differential management practices (Lindqvist *et al.*, 2001; Barger, 1999; 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 (Preston and Allonby, 1987; Abbott *et al.*, 1985; Datta *et al.*, 1999).

The prevalence of *haemonchosis* by localities (provinces) has been investigated in this study. The highest prevalence of infection was in karary (18.2), and East Nile (17.6%), then bahri (12.5%), Omdurman (2.7 %). Whereas the lowest prevalence of infection was in Um bada (0%) and khartoum (0%) and Jabal al Awliya (0%) . There was a significant association between *haemonchosis* and locality (p-value =0.032). This is similar to the

reported results in Ethiopia (Sissay, 2007, and Dagnachew *et al.*, 2011). The reason could be attributed to the geographic location.

In our study sex was investigated. The higher prevalence of infection was in males (6.7%) as compared to females (6.3%). There was no significant association between *haemonchosis* and sex (p-value 0.912). These results are in agreement with the studies carried out in The valley of Kashmir (Irfan *et al.*, 2013) , (Gorski *et al.*,2004) reported that males were more infected with nematode species than females. also our results coincide with other studies Tariq *et al.*, (2010) reported 57.8% prevalence of nematodes in males as compared to 52.7% in female Gulland and Fox (1992) , Gauly *et al.*, (2006) and Tariq *et al.*, (2008) , also observed a little higher percentage prevalence of *H. contortus* in males than female sheep. Tariq *et al.*, (2003) ; Qamar *et al.*, (2009) recorded no significant difference in infection percentage between males and females . Raza *et al.*, (2009) also recorded 34.11% prevalence of *H. contortus* in males and 39.22% in case of females in sheep . Javed *et al.*, (1992) ; Maqsood *et al.*, (1996) ; but Khan *et al.*, (2010) observed more infection in females than males . Therefore, it seems that both sexes are equally susceptible to nematode infection and the differences reported could be the effect of management conditions of the host animals and also may be due to differences in sample size . (Irfan *et al.*, 2013) . Barger (1993) and Bilbo and Nelson (2001) reported that such differential prevalence of gastrointestinal nematodes in sheep may be due to stimulatory effects of estrogen and inhibitory effect of androgens on immune responses. The same factor could be responsible for

the higher prevalence of *H. contortus* in male than female sheep during the present study.

also our results coincide with another study which showed no significant difference of animals of either sex , prevalence in male was 36.71% and in females 37% (Muhammad *et al.*, 2009)

The influence of gender on the susceptibility of animals to parasitic infections could be attributed to genetic predisposition and differential susceptibility owing to hormonal control. Management and climatic conditions also have a greater role to play in the onset of infections.

The results of our studies showed that the prevalence of *haemonchosis* within different age groups of sheep has no significant association (p-value = 0.950). Animals with less than or equal year of age were highly affected (6.7%), compared with animals high than year (6.4%).

The higher infection in young animals than that in younger ones may be attributed to lesser resistance because of lesser exposure time to *haemonchus contortus* in pastures compared with the older animals. These results are in agreement with the studies carried out in Pakistan (Lateef *et al.*, 2005) and Kenya (Ng'an'ga *et al.*, 2004) . also our results coincide with the studies carried out in Pakistan which did not show any significant difference (Qamer *et al.*,2009) and in Ethiopia (Sissay, 2007), (Negasi *et al.*, 2012) and (Dagnachew *et al.*, 2011). this results do not correspond with the studies carried out in Pakistan (Tasawar *et al.*, 2010). Similar results have been reported by Hafeez, (1996) ;

Maqsood et al.,(1996) ; Sajid et al.,(1999) ; Asanji,(1988) ; Dorny *et al.*,(1995) .

The current study showed that the prevalence of *haemonchosis* in different body condition of sheep was : 6.3 % in good body condition, and 10 % in bad body condition. There was no significant association between the body condition and *H. contortus* infection (p-value =0.640) Bad body condition has the highest rate of infection than others with the good condition, because animals in bad body condition have a little tolerance, lack immunity, and therefore more susceptible to infection. This finding agrees with Keyyu *et al.* (2006), Negasi et al. (2012), and Gonfa *et al.* (2013).

In addition, Radostits *et al.* (2006) and Odoi *et al.* (2007) indicated that animals with poor condition are highly susceptible to infection and may be clinically affected by worm burdens too small to harm an otherwise well-fed healthy animal. Moreover, Knox *et al.* (2006) observed that well-fed animal was not in trouble with worms, and usually a poor diet resulted in more helminth infections. Furthermore, helminths also led to a loss of appetite and poor utilization of food, which results in a loss of body weight. Hawkins and Morris (1978) demonstrated that weekly growth rates of wool and live weight decreased with increasing fluke burdens in sheep.

The prevalence of *H. contortus* infection related to breed of animals was 5.7% in Kabashi breed and 3.8 % in Hamarri breed and 25% in dubasy breed . There was significant association between breed and *H. contortus* infection (p-value=0.023). dubasy

breed has a higher rate of infection. This could be attributed to the nature of pasture-grazing patterns of animals, immune response for the parasite, and the topographical location of pasture. These findings are consistent with the observations reported in different breeds of sheep in Pakistan (Tasawar *et al.*, 2010). and also consistent with observations reported in different sheep breed in Pakistan (Chuadary *et al.*, 2007) and disagree with finding of study carried out in Iran which show there is no association between ovine *haemonchosis* and breed (Gerdaghi., 2013), it may be due to genetic variation of world sheep breeds; immunity to helminth parasite was recorded in certain breeds of sheep and goats (Fayza *et al.*, 2003).

Also in this study, the relationship between the prevalence of *haemonchosis* and housing was investigated: The rate of infection was 5.2% when the animals were found indoor compared to 20% in animals kept outdoor. There was a significant association between the *haemonchosis* and housing (p-value=0.026). The higher rate of infection in animals found outdoor. This could be attributed to the fact that probability of infection may increase in animals kept outdoor because it have high chance of exposure to the parasite larvae, under normal condition.

The prevalence of *H. contortus* infection in relation to using anthelmintic was 5.5% in animals whose owners used drugs, 40 % in animals whose owners did not use drugs. There was highly significant association between *H. contortus* infection and using drugs (p-value=0.002). Animals whose owners did not use drugs have a higher rate of infection. This could be attributed to the fact

that using anthelmintic would definitely reduce parasite infection. This finding is in agreement with study carried out in north Kordofan state, Sudan (Mubarak, 2013) and study carried out in Khartoum state (Mohammed, 2014).

Infection was more prevalent in sheep raised with other animals (7%) than the animals that raised alone not with other animals (6.3%). Because may be other animals of the reasons for the spread of the disease, due to the fact that other animals are reservoir of infection. There was no significant association between *H. contortus* infection and presence of other animals (p-value=0.876).

The prevalence of *H. contortus* infection within different fecal consistency of animals was : 0% in animals with soft feces, and 6.7% in animals with normal feces. There was no significant association between *H. contortus* infection and fecal consistency (p-value=0.512).

In this study, the prevalence of *H. contortus* infection in relation to vegetation was investigated. A higher prevalence of infection was in areas of good vegetation (20%) as compared to areas of poor vegetation (5.6%). There was significant association between *haemonchosis* and vegetation area (p-value=0.073).

higher rate of prevalence was found in areas with a good vegetation than poor vegetation areas. This was probably due to the presence of larvae climber in the grass, thus increasing the rate of infection.

The prevalence of *H. contortus* infection in relation to owner knowledge was 2.6 % in animals whose owners were know

about the disease , 14.8% in animals whose owners did not know about disease. There was highly significant association between *H. contortus* infection and owner knowledge (p-value=0.003). Animals whose owners did not know about disease have a higher rate of infection. This could be attributed to the fact that owner's aware of the importance of control and prevention of the disease.

In this study, the prevalence of *H. contortus* infection in relation to source of water was investigated. A higher prevalence of infection was in animal which drink from canal (20%) as compared to animal drink from tape water (5.9%) , and from well 2.7%. there was significant association between the *haemonchus contortus* infection and source of water (p-value =0.065)

This was probably due to fact that the Canals are shallow surface contaminated water may contain a small herbs at the edges. As well as the nature of the land be muddy. These factors all help to spread and growth of L3 and thus spread the disease more.

The results of this study showed that the prevalence of *haemonchosis* in different soil types was 14.7 % in mud soil, and 1% in sand soil. There was highly significant association between the *haemonchus contortus* infection and type of soil (P-value = 0.000).

Conclusion

- The highest prevalence of infection was in Karary (18.2%), and East Nile (17.6%), then Bahri (12.5%), and Omdurman (2.7%). Whereas the lowest prevalence of infection was in Khartoum (0%) and Jabal al Awliya (0%) and Um Bada (0%)
 - A higher prevalence of infection was in males as compared to females.
 - Animals less than or equal 1 year of age were highly affected as compared to more than 1 year.
 - The prevalence of *H. contortus* infection related to breed of animals was higher in Dubasy breed than in Kabashi and then Hamarri breed .
 - High prevalence in poor body condition as compared to good conditions.
 - The rate of infection when the animals were found outdoor was higher than animals found indoor.
 - The prevalence of *H. contortus* infection related to source of water was higher in animal which drink from canal than tap and then well .
- The prevalence was higher in animals having normal faecal consistency than animals having soft fecal consistency .
- The prevalence rate of infection was higher in animals whose owners do not know about the disease , than animals whose owners know.
- .The infection rate of *haemonchus contortus* was higher in animals which their owners not used anthelmintics than animals which their owners used anthelmintics.

- The infection was more prevalent in sheep raised with other animals than the animals raised alone.
- The higher prevalence of infection was in good vegetation as compared to animals grazing in poor vegetation.
- The higher rate of infection was observed in loam soil areas, than in sandy soil areas.

RECCOMINDATIONS

- Awareness of animal owner's about the disease, treatment and the control strategy by de-worm their animals using antihelminthics (Albendazoles , Imidazothiazoles , Ivermectins), on a regular program basis, and other drugs should be raised in order to reduce prevalence of the infection.
- Extensive extension programs should be implemented to make owner aware of the importance of control and /or prevention of the disease, and upgrade knowledge ,skills, linkages between sheep producers and their public and private sectors.
- Improvement of basic animal management systems (housing, watering, control of lambing and kidding times, identification of animal groups most at risk, manure management) as well as grazing management (burning the pasture during the dry season, avoiding muddy and polluted grazing areas).
- Sheep owners are advised to avoid allowing their animal to graze on pasture lands contaminated with *Haemonchus contortus* at the onset of rainfall following a dry spell to reduce the nematode load in susceptible animals.
- Implementation of pasture control to reduce larval contamination and decrease animal density in pasture and at watering points particularly during dry season should be done .

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Appendices

Appendix I

Questionnaire:

Investigation of *sheep haemonchosis* in Khartoum state, Sudan.

Date _____ Locality: _____ Address _____ Herd Code _____

1- Individual risk factors;-

- I- Age ; \leq year (), $>$ year ()
- II- Sex; male (), female ().
- III- Breed; Hamari (), Kabashi (), dubasy ()
- IV- Body condition score ; good (), poor ().
- V- Faecal consistency; normal (), soft ().

2-Management risk Factors;

- I- Housing ; indoor (), outdoor ().
- II- source of water ; Tape (), canal (), well ().
- III- use of anthelmintics ;- Yes (), No ().
- IV- Presence of other animals spp ; Yes (), No ().
- V- owner knowledge ; Yes (), No ().

3-Climatic risk factors;-

- I - Vegetation ;- good (), poor ().
- II- Types of soil ;- mud (), sand ().
- III – Localities ; Bahri (), East Nile (), Omdurman (),
Um bada (), Karary (), Khartoum (), Jabal al Awliya ().

Appendix II

Frequency table for the distribution of infection among 170 sheep examined at Khartoum state according to potential risk factors.

A. Frequency distribution of sex:

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid Female	80	47.1	47.1	47.1
Male	90	52.9	52.9	100
Total	170	100	100	

B. Frequency distribution of age:

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid ≤ year	45	26.5	26.5	26.5
>year	125	73.5	37.5	100
Total	170	100	100	

C. frequency table of body condition:

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid Good	160	94.1	94.1	94.1
Poor	10	5.9	5.9	100
Total	170	100	100	

D. frequency distribution of breed:

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid Kabashy	106	62.4	62.4	62.4
Dubasy	12	7.1	7.1	69.4
Hamary	52	30.6	30.6	100
Total	170	100	100	

E. frequency distribution of housing system:

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid In door	155	91.2	91.2	91.2
Out door	15	8.8	8.8	100
Total	170	100	100	

F. frequency distribution of source of water:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Tape	118	69.4	69.4	69.4
	Canal	15	8.8	8.8	78.2
	Well	37	21.8	21.8	100
	Total	170	100	100	

G. Frequency distribution of using anthelmintic:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	43	25.3	25.3	25.3
	No	127	74.7	74.7	100
	Total	170	100	100	

H. Frequency distribution of owner knowledge :

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	116	68.2	68.2	68.2
	No	54	31.8	31.8	100
	Total	170	100	100	

I. Frequency distribution of other animals :

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	43	25.3	25.3	25.3
	No	127	74.7	74.7	100
	Total	170	100	100	

J. Frequency distribution of faecal consistency:

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid Normal	164	96.5	96.5	96.5
Soft	6	3.5	3.5	100
Total	170	100	100	

K. Frequency distribution of vegetation area:

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid Absent	160	94.1	94.1	94.1
Present	10	5.9	5.9	100
Total	170	100	100	

L. Frequency distribution of Type of Soil:

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid Sand	102	60	60	60
Mud	68	40	40	100
Total	170	100	100	

M. Frequency distribution of animal tested in the localities:

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid Bahri	40	23.5	23.5	23.5
East Nile	17	10	10	33.5
Omdurman	37	21.8	21.8	55.3
Um bada	10	5.9	5.9	61.2
Karary	11	6.5	6.5	67.7
Khartoum	30	17.6	17.6	85.3
Jabal al Awliya	25	14.7	14.7	100
Total	170	100	100	

Appendix III

Cross-tabulation for the distribution of infection among 170 sheep examined at Khartoum state according to potential risk factors investigated.

A. sheep *haemonchosis* and sex cross-tabulation:

Count		Sex		Total
		Female	Male	
Outcome	-ve (%)	75 (75/80x100 =93.8)	84 (84/90x100 =93.3)	159 (159/170x100 =93.5)
	+ve (%)	5 (5/80x100 =6.3)	6 (6/90x100 =6.7)	11 (11/170x100 =6.5)
Total		80	90	170

B. sheep *haemonchosis* and Age cross-tabulation:

Count		Age		Total
		≤ year	>year	
Outcome	-ve (%)	42 (93.3)	117 (93.6)	159 (93.5)
	+ve (%)	3 (6.7)	8 (6.4)	11 (6.5)
Total		45	125	

C. sheep haemonchosis and body condition cross-tabulation:

Count	Body condition		Total
	good	poor	
Outcome			
-ve (%)	150 (93.8)	9 (90)	159 (93.5)
+ve (%)	10 6.3	1 (10)	11 (6.5)
Total	160	10	170

D. sheep haemonchosis and breed cross-tabulation

Count	Breed			Total
	Kabashi	Dubasy	Hamari	
Outcome				
-ve (%)	100 (94.3)	9 (75)	50 (96.2)	159 (93.5)
+ve (%)	6 (5.7)	3 (25)	2 (3.8)	11 (6.5)
Total	106	12	52	170

E. sheep haemonchosis and Housing cross-tabulation:

Count	housing		Total
	In door	Out door	
Outcome			
-ve (%)	147 (94.8)	12 (80)	159 (93.5)
+ve (%)	8 (5.2)	3 (20)	11 (6.5)
Total	155	15	170

F. sheep *haemonchosis* and Source of water cross-tabulation:

Count	Source of water			Total
	Tape water	Canal	Well	
Outcome				
-ve (%)	111 (94.1)	12 (80)	36 (97.3)	159 (93.3)
+ve (%)	7 (5.9)	3 (20)	1 (2.7)	11 (6.5)
Total	118	15	37	170

G. sheep *haemonchosis* and use of anthelmintics cross-tabulation:

Count	use of anthelmintics		Total
	Yes	No	
Outcome			
-ve (%)	156 (94.5)	3 (60)	159 (93.5)
+ve (%)	9 (5.5)	2 (40)	11 (6.5)
Total	165	5	170

H. sheep *haemonchosis* and Owner knowledge cross-tabulation:

Count	Owner knowledge		Total
	Yes	No	
Outcome			
-ve (%)	113 (97.4)	46 (85.2)	159 (93.5)
+ve (%)	3 (2.6)	8 (14.8)	11 (6.5)
Total	116	54	170

I. sheep *haemonchosis* and Other animals cross-tabulation:

Count		Other animals		Total
		Yes	No	
Outcome	-ve (%)	40 (93)	119 (93.7)	159 (93.5)
	+ve (%)	3 (7)	8 (6.3)	11 (6.5)
Total		43	127	170

J. sheep *haemonchosis* and faecal consistency cross-tabulation:

Count		faecal consistency		Total
		normal	soft	
Outcome	-ve (%)	153 (93.3)	6 (4.7)	159 (93.5)
	+ve (%)	11 (6.7)	0 (0)	11 (6.5)
Total		164	6	170

K. sheep *haemonchosis* and Vegetation area cross-tabulation:

Count		Vegetation area		Total
		absent	present	
Outcome	-ve (%)	151 (94.4)	8 (80)	159 (93.5)
	+ve (%)	9 (5.6)	2 (20)	11 (6.5)
Total		160	10	170

L. sheep haemonchosis and type of soil cross-tabulation:

Count	type of soil		Total
	sand	mud	
Outcome			
-ve (%)	101 (99)	58 (85.3)	159 (93.5)
+ve (%)	1 (0.98)	10 (14.7)	11 (6.5)
Total	102	68	170

M. sheep haemonchosis and locality cross-tabulation

Count	Locality							Total
	Bahry	East Nile	Omdurman	Um bada	Karary	Khartoum	Jabal awlia	
Outcome								
-ve (%)	35 (87.5)	14 (82.4)	36 (97.3)	10 (100)	9 (81.8)	30 (100)	25 (100)	159 (93.5)
+ve (%)	5 (12.5)	3 (17.6)	1 (2.7)	0 (0)	2 (18.2)	0 (0)	0 (0)	11 (6.5)
Total	40	17	37	10	11	30	25	170

Appendix IV

Univariate analysis for the association of sheep *haemonchosis* in 170 sheep with potential risk factors using Chi square (χ^2) test.

A. Sex:

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.012	1	.912
Likelihood Ratio	.012	1	.912
Linear-by-Linear Association	.012	1	.912
N of Valid Cases	170		

B. Age:

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.004	1	.950
Likelihood Ratio	.004	1	.950
Linear-by-Linear Association	.004	1	.950
N of Valid Cases	170		

C. Body condition:

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.219	1	.640
Likelihood Ratio	.191	1	.662
Linear-by-Linear Association	.217	1	.641
N of Valid Cases	170		

D. breed :

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	7.515	2	.023
Likelihood Ratio	4.942	2	.085
Linear-by-Linear Association	.030	1	.863
N of Valid Cases	170		

E. housing:

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.976	1	.026
Likelihood Ratio	3.491	1	.062
Linear-by-Linear Association	4.947	1	.026
N of Valid Cases	170		

F. Source of water:

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5.461	2	.065
Likelihood Ratio	4.176	2	.124
Linear-by-Linear Association	.081	1	.776
N of Valid Cases	170		

G. using anthelmintic:

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	9.570	1	.002
Likelihood Ratio	4.919	1	.027
Linear-by-Linear Association	9.513	1	.002
N of Valid Cases	170		

H. Other Animal :

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.024	1	.876
Likelihood Ratio	.024	1	.877
Linear-by-Linear Association	.024	1	.876
N of Valid Cases	170		

I. Vegetation Area :

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.214	1	.073
Likelihood Ratio	2.211	1	.137
Linear-by-Linear Association	3.195	1	.074
N of Valid Cases	170		

J. Owner Knowledge :

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	9.105	1	.003
Likelihood Ratio	8.350	1	.004
Linear-by-Linear Association	9.051	1	.003
N of Valid Cases	170		

K. Type of Soil:

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	12.70	1	.000
Likelihood Ratio	13.47	1	.000
Linear-by-Linear Association	12.62	1	.000
N of Valid Cases	170		

L. Faecal Consistency:

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.430	1	.512
Likelihood Ratio	.818	1	.366
Linear-by-Linear Association	.428	1	.513
N of Valid Cases	170		

M. Localities :

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	13.769	6	.032
Likelihood Ratio	15.895	6	.014
Linear-by-Linear Association	5.928	1	.015
N of Valid Cases	170		