

## **Introduction**

Parasitic diseases pose serious threat to livestock farmers specially sheep and goats keepers. Parasitic diseases of ruminants are linked to the environment; interest in sustainable livestock production provides challenge for those concerned with control and prevention of animal parasitism (Qamar *et al.*, 2011).

The causative agent of Haemonchosis in small ruminant is *Haemonchus contortus*. This parasite 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.*, 2011).

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

Ovine Haemonchosis occurs mainly in lambs and adult sheep could to be affected, the disease occur in three forms: peracute, acute and chronic (Blood and Radostitis, 1989).

### **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 mangemental system in desert and semi desert area. Thus these worms represent a serious problem in sheep industry in the Sudan by this result in reduced the 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 the Sudan may indicated that the preacute could be represent a major problem to sheep industry in the country (Fayza *et al.*, 2003)

Moreover for many years, several methods could used to control this parasite. The use of anthelmintics is the most extensively use to control these nematodes (Getachewet *al.*, 2007).

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

**Objectives:**

- 1- To estimate the prevalence of sheep Haemonchosisin Khartoum state.
- 2- To investigate potential risk factors associated with sheep Haemonchosisin Khartoum state.

## **Chapter One**

### **Literatural Review:**

### 1.1.1 *Haemonchus*:

It is an important parasite of the abomasums of various ruminants. Three species in the genera *Haemonchus* have been identified to have infected camels in different parts of the world. *Haemonchus contortus* occurs in abomasum of camel, sheep, goats and cattle. It is commonly known as the (stomach- worm) or(wire worm) of ruminants. *Haemonchus longistipes* occurs in camel and Arabian camels in North Africa and India. *Haemonchus placei* occurs in the abomasums zof cattle and camels (Falah, 2004). *Haemonchus similis* this species has been reported from cattle and deer in America and also occurs in cattle in Europe (Soulsby, 1982)

### Ovine Haemonchosis:

1.1.2 Etiology: *Haemonchus contortus* - Rudolphi, 1803

### 1.1.3 Classification:

Kingdom: *Animalia*

Phylum: *Nematode* or *Nemathelminths* - Schneider, 1873.

Class: *Secernentea* or *Nematoda* - Rudolphi, 1803.

Order: *Strongylida* - Molin, 1861.

Family: *Trichostrongylidae* - Leiper, 1912.

Genus: *Haemonchus* - Cobb, 1898.

Species: *contortus* -Rudolphi, 1803.

(Solusby,1982;Myers *et al.*, 2013).

### 1.1.4 Morphology:

An adult *Haemonchus contortus* measures about 25 to 34 mm long, the male being shorter than the female which measure about 19 to Twenty two mm, the morphological characteristics of *Haemonchus contortus* are a mouth capsule with a single dorsal lancet and two prominent cervical papillae in the oesophageal

area (Eseta, 2004). 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, 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).

#### **1.1.5 Life cycle of *Haemonchus contortus*:**

The life cycle of *Haemonchus contortus* is direct. The females are prolific egg-layers frequently laying 5000-10,000 eggs a day per female in a few hours, eggs embryonate and hatch into the first stage (L1) larvae. The L1 larvae feed on faecal microflora, develop, grow and moult to the second stage (L2) larvae. After a further period of activity and growth, the third stage (L3) is achieved. The third stage larvae fail to cast off the cuticle of the previous stage and are ensheathed, non-feeding form. Under favorable conditions, the infective stage will be present on the pasture within 4-6 days but may be delayed for weeks or months under cool conditions. The sheathed infective larva is resistant to desiccation and freezing. The larvae disperse from the faecal mass onto herbage, while moisture is adequate; they survive until they invade the host. Infection of trichostrongylids is usually via the oral route. After ingestion, the larvae exsheath in the rumen and migrate to abomasums, the actual stimuli to exsheathment are unknown but are thought to be dissolved carbon dioxide and / or undissociated carbonic acid in the gut. The parasitic exsheathed L3 migrate to the abomasum and become closely associated with the mucosa, where the third moult occurs and the fourth stage (L4) larvae emerge. The L4 is able to feed once the L3 sheath is lost and, just

before the time of the fourth moult, the piercing lancet which enables the larvae to penetrate the surface of the abomasal mucosa develops. Feeding commences and is soon followed by the fourth moult to the fifth or pre-adult stage. After further feeding, the fifth stage larvae mature into adult worms which are to be found moving freely on the surface of the mucosa. Differentiation into male and female begins around the time of the fourth moult. They reach maturity in 15 days, the first eggs appearing in the faeces of the host about 15 days after infection (Rugutt, 1999).

#### **1.1.6 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 unfavourable 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. Other 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).

#### **1.2.1 Pathogenesis:**

Several factors are involved in the pathogenesis of the Haemonchosis. In terms of the development of disease, the most important factors are parasites virulence and host response. The main pathogenic mechanism of *Haemonchus*

*contortus* are a direct lesion on the gastric mucosa and hematophagy. The effects of pathogenic mechanisms during intra-host parasite development and the subsequent response of infected ruminants provoke morpho-functional changes, particularly in the abomasums; also variation appears in some blood parameters, resulting in the appearance of both anemic and impaired digestion - absorption syndrome. *Haemonchus contortus* adult parasites can ingest 0.05 ml of blood per helminth per day. Causing notable blood loss with a reduction of packet cell volume (PCV). This parameter has, in fact been used a marker of parasite virulence and indirect estimation of parasite burden in Haemonchosis (Francisco, 2007).

A reduction of plasma protein concentration has been found in Haemonchosis due to blood loss and haemorrhagic gastritis, in addition leakage of proteins to gastric lumen occur as a result of disruption of intercellular unions and increased permeability, epithelial cell loss, tissue reparation, increase in mucus production and increased requirements for protein synthesis by the immune system(Francisco, 2007).

Moreover, many factors prevent such protein losses from being replaced through feeding. Infected animals have lower food intake due to anemia. Gastric reduces food passage through gastrointestinal tract, bad digestion syndrome. Caused by the increase of abomasal P.H value, which prevent pepsin synthesis. Reduces aminoacids and small peptides absorption (Francisco, 2007).

### **1.2.2 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).

In goats; the mixed infection with *Haemonchus contortus* and *Eimeria species* showed; the abomasae of infected animals were congested and the intestine were thick. The spleen, liver, intestines and kidneys were ischemic. Moreover, petechial hemorrhage and purulent exudates was seen in the bronchioles and lobules of the lungs, gelatinous exudates was seen in the interlobular septate (Siham *et al.*, 1997)

### **1.3 Immunology:**

#### **1.3.1 Immune response to *Haemonchus contortus*:**

Both humoral and cellular arms of the mammalian adaptive immune system are actively involved in response to Haemonchosis, generally, T-lymphocytes, soluble cytokines, B-lymphocytes, plasma cells, various immunoglobulin isotypes, mast cells, eosinophils and globule leukocytes are known to actively take part in immunological reactions, although variability in their production and magnitude of action in different species of parasite and host has been observed. The ultimate result of parasitic invasion of a host animal is either establishment of infection or expulsion of the invading parasite. The latter being the consequence of protective immune response of the host (Krishna, 2007).

#### **1.3.2 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). After being ingested, L3 start the process of growth and development which includes exsheathing, molting from one stage to another, and shedding excretory and secretory products, during this process antigens are shed in the gastrointestinal tract and are presented by epithelial cells to underlying gut associated lymphoid tissue. Presentation of parasitic antigens are transported by M cells to the antigen specific T and or B cells in the Peyer's patches which is followed by a cascade of cellular and subcellular activities such as activation of antigen specific T and or B cells, production of a variety of cytokines that bring about activation of various cells like eosinophils, mast cells, macrophages and globule leukocytes. In addition, production of different immunoglobulin isotypes brings about immune responses leading to expulsion of worms and protection against re-infection (Krishna, 2007).

#### **1.4 Clinical manifestations and Signs:**

Haemonchosis in sheep may be classified as hyperacute, acute, or chronic. In the hyperacute 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 characterised 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 ascalreticulin, enabling the parasite to feed easily on host blood (Getachew *et al.*, 2007).

In the dual infections of lambs with *Haemonchus contortus* and *Eimeria spp.*, sever scouring was noticed, weight loss and high mortality rates presented additional manifestations (Abakar *et al.*, 2001)

### **1.5 Advanced diagnosis:**

#### **1- Serological diagnosis :**

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 immune affinity purified somatic antigen could detect infection as early as one week post infection during pre-patency (Durrani *et al.*, 2007; Lone *et al.*, 2012).

#### **2- 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 ( $\beta$ -actin, IFN- $\gamma$ , TNF- $\alpha$ , 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 µg/ml, 24 hours in a 5% CO<sub>2</sub> and 37°C atmosphere) was then performed (Getachew *et al.*, 2007).

### **3- Immunological diagnosis:**

#### **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 faeces 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 faecal 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).

## **1.6 Treatment of Haemonchosis:**

### **1.6.1 Broad-spectrum anthelmintics:**

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

#### **Group 1: 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 it is effective. 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: Levamisole (LM) ('yellow' drenches)**

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

### **Group 3: Macrocyclic lactones ('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<sup>-</sup> channels and  $\gamma$ -aminobutyric acid (GABA) neurotransmission sites in nematodes, blocking interneuronal stimulation of inhibitory motor neurones, leading to a flaccid paralysis (Abbott *et al.*, 2009).

#### **1.6.2 Narrow spectrum anthelmintics:**

The substituted phenols (nitroxylin) 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, and 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.*, 2010).

**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.*, 2010).

### **1.7 Some control strategy:**

#### **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).

#### **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).

### **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 to 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).

**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 re-growth 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).

## **1.8 Epidemiology:**

### **1.8.1 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 and Waller 2005).

### **1.8.2 Prevalence of sheep Haemonchosis:**

In a study 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).

A total of 498 faecal 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).

Another study examined gastrointestinal tracts of 79 sheep and 161 goats 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 spp. (99.8%) and *monezia expansa* (0.2%) in sheep beside eight nematodes spp. (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 colabrifformis* (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 colubrifformis*(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 an epidemiological study 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 two-year epidemiological study of helminths of small ruminants involved the collection of viscera from 655 sheep and 632 goats from 4 abattoirs in Eastern

Ethiopia. A further more detailed epidemiological study of gastro-intestinal nematode infections used the Haramaya University (HU) flock of 60 black head Ogaden sheep. The parasitological data included numbers of nematode eggs per gram of faeces (EPG), faecal culture L3 larvae, packed red cell volume (PCV), adult worm and early L4 counts, and FAMACHA eye-colour score estimates, along with animal performance (body weight change). There were 13 species of nematodes and 4 species of flukes present in the sheep and goats, with *Haemonchus contortus* being the most prevalent (65–80%), followed by *Trichostrongylus* spp. The nematode infection levels of both sheep and goats followed the bi-modal annual rainfall pattern, with the highest worm burdens occurring during the two rain seasons (peaks in May and September). There were significant differences in worm burdens between the 4 geographic locations for both sheep and goats. Similar seasonal but not geographical variations occurred in the prevalence of flukes. There were significant correlations between EPG and PCV, EPG and FAMACHA scores of anemia, and PCV and FAMACHA scores. Moreover, *Haemonchus contortus* showed an increased propensity for arrested development during the dry seasons (Sissay, 2007).

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 abomasums, 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 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 spp.* 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 spp.* in a single small ruminant host, suggesting occurrence of *Haemonchus spp.*, circulation among heterologous hosts sharing the same pastures that should be considered in the control strategy of the parasite (Kumsa and Wossene, 2006).

From August 2004 to December 2005, a total of 338 faecal samples were collected (86 from sheep 252 from goats) to determine the prevalence of various endoparasites in and around twin cities of Rawalpindi and Islamabad, of the total samples examined, 65.7% were found positive for endoparasites. The prevalence of gastrointestinal parasites tended to be higher ( $p = 0.059$ ) in sheep 62 (72%) than in goats 160 (63.7%). The endoparasites 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).

A study was carried out in 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 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).

Another 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).

An investigation 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 spp.*, 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 (Garedaghi *et al.*,2013).

From August 2004 to May 2008, a total of 400 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 endoparasites. 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 previous study was conducted to determine 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 (Ngaga *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 contortus*, *Trichostrongylus axei*, *T.colubriformis*, *Ostertagia*

*trifurcata*, *Ostertagia circumcincta* and *Cooperia 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 spp.* (46.1%), *Ostertagia* (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).

A study was carried out to investigate the prevalence and seasonal trend of the *Haemonchus contortus* in sheep and goats in the Potohar areas of Northern Punjab, Pakistan from December 2004 to January 2006. Faecal samples collected from 968 sheep and 961 goats of different breeds were examined by the modified McMaster technique using saturated solution of sodium chloride. Results revealed that the infection was significantly ( $P < 0.05$ ) higher in sheep compared to goats. The peak infection level was recorded during rainy season (July-October). On the other hand, low infection level was noted from December up to May. In sheep, highest log faecal egg counts (LFECs) were recorded in Islamabad, followed by Attock, Jhelum and Chakwal. However, in goats the LFECs trend was highest in Islamabad, followed by Jhelum, Attock and Chakwal districts. A significant ( $P < 0.05$ ) variability in LFECs was noted between sheep and goat breeds from site-site, while no significant difference was observed between breeds at the same site. Hairy (Jattal) goats and salt-range (Latti) sheep breeds



exhibited significantly reduced LFECs level along with higher packed cell volume (PCV) and haemoglobin (Hb) levels compared to other breeds. Moreover, FAMACHA © chart scoring in relation with worm infection (FECs) was more valid in sheep than goats. High prevalence of *Haemonchus contortus* in Potohar areas was due to favorable agro-climatic conditions that favor the development and survival of the free-living stages of *Haemonchus contortus*. The findings are discussed with regard to their relevance for strategic control of Haemonchosis in small ruminants (Chaudary *et al.*,2007).

Another study carried out to assess the prevalence of gastrointestinal helminths infections among wild and domestic ruminants in Cholistan desert of Pakistan. For this purpose, 1010 faecal samples of different species of ruminants including cattle (n=300), sheep (n=250), goat (n=100), camel (n=200), chinkara (n=150) and black buck (n=10) were examined using standard parasitological procedures. The highest prevalence was recorded in cattle (44.7%) followed by sheep (43.6%), goats (39%), camels (37%), chinkara (26.7%) and black bucks (20%). Maximum number of the helminth species were recorded in sheep (n=14) followed by camels (n=13), cattle (n=09), goats (n=08), chinkara (n=07) and black bucks (n=02), Nematodes were the predominantly occurring (n=18) helminths followed by Trematodes (n=6) and Cestodes (n=3). *Haemonchus* and *Trichostrongylus* were the most frequently recorded genera followed by *Chabertia*, *Oesophagostomum*, *Schistosoma*, *Moniezia*, *Cooperia*, *Bunnostomum*, *Toxocara*, *Ostertagia*, *Nematodirus*, *Trichuris*, *Strongyliodes*, *Avitellina*, *Fasciola*, *Thelazia* (n=02), *Syngamus*, *Gaigeria*, *Skrjabinema*, *Cotylophoron*, *Metastrongylus* and *Gongylonema* as mixed or single species infections in different species of animals. It was concluded that wild and domesticated ruminants of the Cholistan desert of Pakistan suffer with heavy infections of a variety of helminths including those of high economic significance.

Therefore, high prevalence of helminthes warrants immediate attention of the stakeholders for devising an effective worm control program in the Cholistan desert (Zahid *et al.*,2012).

A survey was conducted to determine the prevalence of *Haemonchus contortus* in slaughtered sheep at Urmia abattoir located in the Northwest 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 spp.*, 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 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 (Fentahunand Girija,2012).

An epidemiological 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, and 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).

A pervious study was conducted to compare prevalence of infections with flukes, tape worms and nematodes parasitizing gastrointestinal tract in small ruminants from various regions of District Ganderbal Kashmir. Visceral examinations of 284 sheep and 318 goats indicated a marked variation in the level of parasitism in animals raised in different geographic areas. Prevalent nematodes were *Haemonchus* (82%), *Trichuris* (74%), *Nematodirus* (60%), *Trichostrongylus* (58%), *Chabertia* (52%), *Strongyloides* (42%) and *Oesophagostomum* (46%). Among cestodes, *Moneizia*(48%), *Avitellina* (42%) and *Thysenezia* (28%) were reported. Among trematodes, *Fasciola* (60%), *Dicrocoelium* (52%) and, *Paramphistomum* (46%) were most prevalent. The study indicates the prevalence of gastrointestinal helminthic infections varies in different seasons and in different age groups (Basher *et al.*, 2012).

An epidemiological survey of prevalence of Haemonchosis in sheep was conducted in Jammu area of Jammu And Kashmir State. A total of 257 animals were examined of which 61 (23.73%) were found positive for *Haemonchus contortus* as revealed by necroscopic examination. The infection was maximum in spring and summer and lower in winter and autumn. Lower age groups were having more infection (36.48%). Males were found to harbor more infection (27.43%) than females (20.83%). Exotic breeds were found to have more infection than local ones(Muzaffaret *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, we divided Khartoum state into three towns Khartoum, Khartoum North and Omdurman. In the second, level, Omdurman, Um bada, Khartoum and East Nile localities were randomly selected. In the third level, the study areas were selected according to the presence of sheep within the locality. The distribution of the sample in the localities as the following: 40 animal from Omdurman, 165 animal from East Nile, 18 animal from Um bada and 17 animal from Khartoum locality. From Omdurman: Soug Elzreiba, Elsoug Elshabei and Soug Elmowielh, from Um bada: Elredwan animals complex, Soug Elmashia and Soug Elsalam, from East Nile: Hilat Kuku, Elshigela, Elkeryab, Zrayeb Elnasr, Eid babiker and Soba sherg ,While from Khartoum: Elremailah and Soug Elsahafa ; these areas were selected randomly.

## **2.3 Sample size:**

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

$$n = \frac{1.96^2 (P^{\wedge})(Q^{\wedge})}{L^2}$$

n = sample size

(1.96)<sup>2</sup>= constant

P<sup>^</sup> = expected prevalence

Q<sup>^</sup> = 1-P

L = allowable error

The previous prevalence selected equals 28% according to Nganga, in a study conducted in a semi-arid area in Kenya(Nganga et al.,2004). The confidence level was chosen as 95% and the allowable error equal 5%;

$$n = \frac{4 \times 28 \times (27\%)^2}{25} = 120.96$$

Accordingly the sample size calculated was 120 animals. This sample size was inflated by two to increase precision of the result (Thursfield, 2007).

#### **2.4 Animals sampled:**

The total animals which sampled was 240 animals, 90 animals of them were Hamari breed, 45 animals were Kabashi breed and 105 animals were Ashgar breed. One hundred twenty seven animals were female while 113 animals were males. Fifty four animals were aged less than 1 year, 124 animals were aged between 1-3 years and 62 animals aged more than 3 years.

#### **2.5 Sample collection and laboratory diagnosis:**

### **2.5.1 Faecal collection:**

In the last of dry and the beginning of wet season, faecal samples were collected directly from the rectum weekly in plastic container and transported to laboratories for diagnosis.

### **2.5.2 Faecal analysis and egg counts (eggs / gram of faeces):**

Rectal faecal samples from all sheep were collected manually in plastic containers and were carefully labeled with animal identification, age, sex and date of collection. Samples were prepared for identification of *Haemonchus contortus* eggs in saturated NaCl solution. Eggs per gram (EPG) of fecal sample were counted to estimate the worm burden using Mc master technique (Maposa, 2009).

#### **Mc master egg counting technique:**

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

#### **Faecal samples culture and Baermann technique:**

Female nematodes of the superfamilies Strongyloidea and Trichostrongyloidea produce typical Strongyle egg that is a smooth surfaced, ellipsoidal egg with embryo in the morula stage of development. It is therefore



difficult to establish the generic or species identify of eggs of these important parasites by microscopic inspection, to obtain an accurate diagnosis of the species involved in an infection with such parasites, it is necessary to examined the third stage larvae (L3) by culturing the faecal sample by providing a suitable environmental for the hatching and development of these eggs to the infective L3 stage. These larvae can be recovered by Baermann technique (Lynda *et al.*, 2011).

### **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. The entire faecal sample was incubated at 27<sup>0</sup>c for 10-14 days, and the fecal culture was starved daily and examined.

### **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 10grams 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 collect it by the gravitation in the stem of the funnel. A few milliliters of the fluid in the stem were 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:**

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 adding to fix the larvae; the nematode larvae stain dark brown (MAFF, 1986 and Lynda *et al.*, 2011).

### **Characters used for identification:**

The identification of *Haemonchus* third stage larvae was carried out according to Lynda *et al.*, (2011) based on the morphological features:

- Have a kinked sheath.
- Have a rounded head.
- Have a pointed tail.
- Medium size of tail sheath



Figure

1: Strongyle egg (Chaney,2012).



Figure 2: 3<sup>rd</sup> stage (L3) *Haemonchus contortus* larva(Chaney,2012).

## 2.6 Questionnaire survey:

Information about potential risk factors suspected to be associated with Haemonchosis were collected from owners and/or managers of all farms involved in the study. The questionnaire included information about breed, age, sex, body condition score, other diseases, animal source, and fecal consistency as individual risk factors. Also herd size, housing, type of grazing, using of anthelmintics, presence of other animals were included as mangemental risk factors. Also, the season, vegetation, temperature, humidity, rainfall and type of soil were included as climatic risk factors, the Meteorological data was obtained from the

Meteorological Authority. These potential risk factors were divided into categories (Thrusfield, 2007).

## **2.7 Data analysis:**

The data collected were entered into computer on Microsoft 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  $\leq 0.25$  (two tailed;  $\alpha = 0.25$ ) were considered significant in the  $\chi^2$  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 Prevalence:**

A total of 240 sheep were tested by count of egg per gram (EPG) and larval identification by fecal culture using Baerman technique. Twenty nine animals were found positive(12.1%) and 131 animals were found negative (87.9%) for sheep Haemonchosis (diagnosed by egg count and confirmed by

larval identification) (Table:3.1).Therefore the overall prevalence of sheep Haemonchosis in Khartoum state was 12.1%.

**Table 3.1:**

**Frequency table for the prevalence of *Haemonchus contortus* in 240 sheep from Khartoum, state diagnosed by faecal egg count and larval identification by Baerman technique**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	-ve	211	87.9	87.9	87.9
	+ve	29	12.1	12.1	100
	Total	240	100	100	

### 3.2 Localities:

The results showed that the overall prevalence of Haemonchosis out of 240 examined sheep in Khartoum state was 12.1%, the highest rate of infection was in Um bada (22.2%), and East Nile (12.7%). Whereas the lowest rate of infection was in Khartoum (5.9%) (Table 3.2) and (Table 3.3).

The Chi square test, showed that there was no significant association between *Haemonchus contortus* infection and locality (p-value= 0.360) (Table 3.4).

### 3.3 Age of animals:

The results of study showed the distribution of 240 sheep examined for Haemonchosis by age. Total number less than 1 year of age was 54 animals. Among these, 2 animals were found infected. The rate of infection was 3.7%. Total number of animals 1-3 years of age was 124 animals. Among these, 11 animals were found infected. The rate of infection was 8.9%. Total number of animals more than 3 years was 62 animals. Among these, 16 animals were found infected. The rate of infection was 25.8% (Table 3.2 and Table 3.3).

In the Chi-square test, the result showed that there was significant association between *Haemonchus contortus* infection and age of animal (p-value = 0.000) (Table 3.4).

### **3.4 Sex of animals:**

The results of study showed the distribution of 240 sheep examined for Haemonchosis by sex. Total number of female examined was 127 animals. Among these, 18 animals were found infected. The rate of infection was 14.2%. Total number of males examined was 113, among these, 11 animals were found infected. The rate of infection was 9.7% (Table 3.2 and Table 3.3).

The Chi-square test, showed that there was no significant association between *Haemonchus contortus* infection and sex of animal (p-value = 0.292) (Table 3.4).

### **3.5 Breed of animals:**

The results of study showed distribution of *Haemonchus contortus* infection in Khartoum state by breed. The number of Hamarri breed examined was 90 animal, among these, 7 animals was found infected, the rate of infection was 8%. The number of Kabashi breed examined was 45 animals. Among these 12 animals were found infected. The rate of infection was 26.7%. While number of Ashgar

breed examined was 105 animals. Among these 10 animals were found infected. The rate of infection was 9.5% (Table 3.2 and Table 3.3).

The Chi square test showed there was significant association between *Haemonchus contortus* infection and breed (p-value= 0.004) (Table 3.4).

### **3.6 Body condition of animal:**

The body condition of the animals and presence of *Haemonchus contortus* have been investigated. One hundred fifty one animals were found in good condition. Among these 17 animals were found infected. The rate of infection was 11.3%, While 88 animals in moderate condition. Among these 11 animals were found infected. The rate of infection was 12.5%, and 2 animal in bad condition, one of them was positive and the rate of infection was 50 %.( Table 3.2 and Table 3.3).

The Chi square test showed that there was no significant association between *Haemonchus contortus* infection and body condition (p-value=0.247) (Table 3.4).

### **.37 Faecal consistency:**

The results of study showed the distribution of *Haemonchus contortus* infection in Khartoum by faecal consistency. Animals with normal faeces were 189 animals. Twelve animals out of them were found infected. The rate of infection was 6.3%. Animals with soft faeces were 50 animals. Among them, 16 animals were found infected. The rate of infection was 32%. Animals with diarrhoea were 2, one of them was positive. The rate of infection was 50% (Table 3.2 and Table 3.3).



The Chi square test showed that there was significant association between *Haemonchus contortus* infection and faecal consistency (p-value= 0.000) (Table 3.4).

### **3.8 Source of animals addition to the herd:**

Source of animal addition to the herd and the presence of *Haemonchus contortus* infection have been investigated. One hundred eighty seven animals were raised on farm. Among them 20 animals were found infected with infection rate of 10.7%. While 28 animals in farms that purchased additions from other farms. Among them, 4 animals were found infected; the rate of infection was 14.3%. While 25 animals raised in farms that purchased additions from local Markets, the rate of infection was 20% (Table 3.2 and Table 3.3).

The Chi-square test showed that there was no significant association between *Haemonchus contortus* infection and source of animals addition to the herd (p-value 0.379) (Table 3.4).

### **3.9 Animal herd size:**

Herd size and the presence of *Haemonchus contortus* infection have been investigated. The number of sheep examined in the category (1-35) animals was 62 animal. Among these, 9 were found infected. The rate of infection was 14.5%. The number of animals examined in the category 36-70 animal was 123 animal. Among these, 15 animals were found infected. The rate of infection was 12.2%. The number of animals examined in the category more than 70 animal was 55 animals. Among these, 5 animals were found infected. The rate of infection was 9.1% (Table 3.2 and Table 3.3).

The Chi square test showed that there was no significant association between *Haemonchus contortus* infection and herd size (p-value= 0.667) (Table 3.4).

### **3.10 Housing:**

Two hundred forty sheep of various housing types were examined in this study, and the presence of *Haemonchus contortus* was investigated. Ninety three of sheep raised indoor were examined, among these, 12 animals were found infected. The rate of infection was 12.9%. While 147 of sheep raised outdoor were examined. Among these, 17 animals were found infected. The rate of infection was 11.6% (Table 3.2 and Table 3.3).

The Chi square test showed that there was no significant association between *Haemonchus contortus* infection and housing (p-value 0.757) (Table 3.4).

### **3.11 Grazing type:**

Two hundred forty sheep of various grazing type were examined in this study. The presence of *Haemonchus contortus* in various grazing type was investigated. Eighty one sheep were in the category Zero grazing. Among these, 16 animals were found infected. The rate of infection was 19.7%. While 159 of sheep were in semi nomadic system. Among these, 13 animals were found infected. The rate of infection within semi nomadic system was 8.2% (Table 3.2 and Table 3.3)

The Chi square test showed that there was significant association between *Haemonchus contortus* infection and grazing type (p-value=0.009) (Table 3.4).

### **3.12 Use of anthelmintics:**

Two hundred forty sheep categorized according to using of anthelmintics, and the presence of *Haemonchus contortus* infection was investigated. Total animals number in the category of anthelmintics used was 153 animals. Among these, 10 animals were found infected. The rate of infection was 6.5%. While 87 animals in the category of anthelmintics not used. Among these; 19 animals were found infected. The rate of infection was 21.8% (Table 3.2 and Table 3.3).

The Chi square test showed that there was significant association between *Haemonchus contortus* infection and using of anthelmintics (p-value= 0.000) (Table 3.4).

### **3.13 Other diseases status:**

The study results showed the distribution of *Haemonchus contortus* infection in Khartoum state by the other diseases status. Total sheep number examined which show absence of any other clinical diseases was 220 animals. Among these, 25 were found infected. The rate of infection was 11.4%. Total sheep number examined which show other clinical diseases were 20 animals. Among these, 4 animals were found infected. The rate of infection was 20% (Table 3.2 and Table 3.3).

The Chi square test showed that there was no significant association between *Haemonchus contortus* infection and other diseases (p-value 0.257) (Table 3.4).

### **3.14 Other animals:**

The study results showed the distribution of *Haemonchus contortus* infection in Khartoum by presence of other animal's species with the sheep in the same farm. Total numbers of sheep which raised alone were 199 animals. Among these, 21 animals were found infected. The rate of infection was 10.6%. Sheep

raised in the presence of other animal's species were 41 animals. Among these, 8 animals were found infected. The rate of infection was 19.5% (Table 3.2 and Table 3.3).

The Chi square test showed that there was no significant association between the *Haemonchus contortus* infection and present of other animals (p-value= 0.109) (Table 3.4).

### **3.15 Season:**

Ovine Haemonchosis in various seasons of the year was investigated. Total number of sheep examined during the dry season was 185 animals. Among these, 19 animals were found infected. The rate of infection was 10.3%. While 55 animals were examined during the wet season. Among these, 10 animals were found infected. The rate of infection was 18.2% (Table 3.2 and Table 3.3).

The Chi-square test showed that there was no significant association between *Haemonchous contortus* infection and season (p-value 0.114) (Table 3.4).

### **3.16 Types of soil:**

Two hundred forty sheep raised on various types of soil were examined for *Haemonchus contortus* infection. Total number of sheep examined which raised in mud soil was 180 animals. Among these, 19 animals were found infected. The rate of infection was 10.6%. While 60 of animals which examined raised in sand soil. Among these, 10 animals were found infected. The rate of infection was 16.7% (Table 3.2 and Table 3.3).

The Chi square test showed that there was no significant association between *Haemonchus contortus* infection and type of soil (p-value =0.208) (Table 3.4).

### **3.17 Rainfall:**

Two hundred forty sheep were examined for *Haemonchus contortus* infection in various rainfall statuses. Total number of animals examined in the absent of rainfall was 185 animals. Among these, 19 animals were found infected. The rate of infection was 10.3%. While number of sheep examined in the present of rainfall was 55 animals. Among these, 10 animals were found infected. The rate of infection was 18.2% (Table 3.2 and Table 3.3).

The Chi square test showed that there was no significant association between *Haemonchus contortus* infection and rainfall status (p-value 0.114) (Table 3.4).

### **3.18 Vegetation:**

The study results showed the distribution of *Haemonchus contortus* infection in Khartoum state by vegetation condition. The number of sheep examined in the dry areas was 71 animals. Among these, 6 animals were found infected. The rate of infection was 8.5%. Whereas the number of sheep in poor vegetative areas was 169 animals. Among these, 23 animals were found infected. The rate of infection was 13.6% (Table 3.2 and Table 3.3).

The Chi square test showed that there was no significant association between the *Haemonchus contortus* infection and vegetation condition (p-value = 0.263) (Table 3.4).

### **3.19 Temperature:**

The study results showed the distribution of *Haemonchus contortus* infection in Khartoum state by temperature. Animals examined in areas with a temperature more than 40°C were 69 animals. Among these, 10 animals were found infected. The rate of infection was 14.5%, while animals examined in areas with a temperature less than 40°C were 171 animals. Among these, 19 animals were found infected. The rate of infection was 11.1 % (Table 3.2 and Table 3.3).

The Chi square test showed that there was no significant association between *Haemonchus contortus* infection and Temperature (p-value 0.467) (Table 3.4).

### **3. 20 Humidity:**

The results showed the distribution of *Haemonchus contortus* infection in Khartoum state by humidity. Total number of animals examined at humidity more than 40% was 69 animals. Among these, 10 animals were found infected. The rate of infection was 14.5%. While animal's number examined at humidity less than 40% was 171 animals. Among these, 46 animals were found infected. The rate of infection was 11.1%. (Table 3.2 and Table 3.3).

The Chi square test showed that there was no significant association between *Haemonchus contortus* infection and humidity (p-value 0.467) (Table 3.4).

### **Results of multivariate analysis:**

Eleven potential risk factors were found to be significantly (P-value  $\leq 0.25$ ) associated with sheep Haemonchosis in the univariate analysis (Table 3.4) entered to logistic regression; final model, out of these eleven risk factors, five risk factors were found significantly (P-value  $\leq 0.05$ ) associated with sheep

Haemonchosis in the multivariate analysis. These five risk factors included: age of sheep(P-value = 0.000), breed of sheep(P-value = 0.004), faecal consistency (P-value = 0.000), grazing type (P-value = 0.009) and use of anthelmintics (P-value = 0.000).

**Table 3.2:** Summary frequency for the distribution of 240 sheep examined for Haemonchosis by count of egg per gram (epg) and faecal culture according to potential risk factors investigated

Risk factor	Frequency	Relative frequency (%)	Cumulative frequency (%)
Age :			
<1 yr	54	22.5	22.5
1-3 yr	124	51.7	74.2
> 3 yr	62	25.8	100
Sex :			
Male	127	52.9	52.9

Female	113	47.1	100
Breed:			
Ashgar	105	43.8	43.8
Hamari	90	37.5	81.3
Kabashi	45	18.7	100
Body condition:			
Good	150	62.5	62.5
Moderate	88	36.7	99.2
Poor	2	0.8	100
Fecal consistency:			
Normal	188	78.3	78.3
Soft	50	20.8	99.2
Diarrohea	2	0.8	100
Source of animals additions:			
Raised on farm	187	77.9	77.9
Purchase from farms	28	11.7	89.6
Purchase from markets	25	10.4	100
Herd size:			
1-35	62	25.8	25.8
36-70	123	51.3	77.1
>70	55	22.9	100
Housing:			
Indoor	147	61.2	61.2
Outdoor	93	38.8	100

Table 3.2 continued

Risk factor	Frequency	Relative frequency	Cumulative frequency
Grazing:			
Zero grazing	81	33.8	33.8
Semi nomadic	159	66.2	100
Use of anthelmintics:			
Yes	153	63.8	63.8
No	87	36.2	100
Other disease:			
No	220	91.7	91.7



Yes	20	8.3	100
Other animal:			
No	199	82.9	82.9
Yes	41	17.1	100
Season:			
Dry	185	77.1	77.1
Wet	55	22.9	100
Type of soil:			
Mud	180	75	75
Sand	60	25	100
Rainfall:			
Absent	195	77.1	77.1
Present	55	22.9	100
Vegetation:			
Absent	71	29.6	29.6
Poor	169	70.4	100
Temperature:			
<40	171	71.3	71.3
> 40	69	28.7	100
Humidity:			
<40	171	71.3	71.3
> 40	69	100	100
Locality:			
Omdurman	40	16.7	16.7
Khartoum	17	7.1	23.8
Um bada	18	7.5	31.3
East Nile	165	68.8	100

**Table 3.3:** Summary cross-tabulation of Haemonchosis in 240 sheep examined by count of egg per gram (epg) and faecal culture according to potential risk factors investigated

Affected %	Animals affected	Animals tested	Risk factors
3.7	2	54	Age:
8.9	11	124	<1 yr
25.8	16	62	1-3 year
			> 3 years
9.7	11	113	Sex:
			Male

14.2	18	127	Female
9.5	10	105	Breed:
8	7	90	Ashgar
26.7	12	45	Hamari
			Kabashi
11.3	17	150	Body condition:
12.5	11	88	Good
50	1	2	Moderate
			Poor
6.4	12	188	Fecal consistency:
32	16	50	Normal
50	1	2	Soft
			Diarrhea
10.7	20	187	Source of animals additions:
14.3	4	28	Raised on farm
20	5	25	Purchase from farm
			Purchase from markets
14.5	9	62	Herd size:
12.2	15	123	1-35
9.1	5	55	36-70
			>70
12.9	12	93	Housing:
11.6	17	147	Indoor
			Outdoor
19.7	16	81	Grazing:
8.2	13	159	Zero grazing
			Semi nomadic

Table 3.3 continued

Affected %	Animals affected	Animals tested	Risk factor
6.5	10	153	Use of anthelmintics:
21.8	19	87	Yes
			No
11.4	25	220	Other disease:
20	4	20	No
			Yes

10.6 19.5	21 8	199 41	Other animal: No Yes
10.3 18.2	19 10	185 55	Season: Dry Wet
10.6 16.7	19 10	180 60	Type of soil: Mud Sand
10.3 18.2	19 10	185 55	Rainfall: Absent Present
8.5 13.6	6 23	71 169	Vegetation: Absent Poor
11.1 14.5	19 10	171 69	Temperature(c <sup>0</sup> ): < 40 > 40
11.1 14.5	19 10	171 69	Humidity (%): < 40 > 40
7.5 5.9 22.2 12.7	3 1 4 21	40 17 18 165	Locality: Omdurman Khartoum Um bada East Nile

Table 3.4 : Summary of univariate analysis for risk factors associated with sheep Haemonchosis in Khartoum state, Sudan (June 2013 to August 2013 ; n=240) using the Chi-square test

<i>p- value</i>	$\chi^2$	Df	Animals affected (%)	Animals tested	Risk factors
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0.000	15.7	2	2 (3.7) 11 (8.9) 16 (25.8)	54 124 62	Age: < 1 yr 1-3 year > 3year
0.292	1.1	1	11 (9.7) 18 (14.2)	113 127	Sex: Male Female
0.004	11.2	2	10 (9.5) 7 (8) 12 (26.7)	105 90 45	Breed: Ashgar Hamari Kabashi
0.247	2.8	2	17 (11.3) 11 (12.5) 1 (50)	151 88 2	Body condition: Good Moderate Poor
0.000	27.1	2	12 (6.3) 16 (32) 1 (50)	189 50 2	Fecal consistency: Normal Soft Diarrhea
0.379	1.9	2	20 (10.7) 4 (14.3) 5 (20)	187 28 25	Source of animal additions Raised on farm Purchase from farms Purchase from markets
0.667	0.81	2	9 (14.5) 15 (12.2) 5 (9.1)	62 123 55	Herd size: 1-35 36-70 >70
0.757	0.09	1	12 (12.9) 17 (11.6)	93 147	Housing: Indoor Outdoor
0.009	6.7	1	16 (19.7) 13 (8.2)	81 159	Grazing: Zero grazing Semi nomadic

Table 3.4 continued

P- value	$\chi^2$	Df	Animals affected (%)	Animals tested	Risk factor
0.000	12.2	1	10 (6.5) 19 (21.8)	153 87	Use of Anthelmintics: Yes No
0.257	1.2	1	25 (11.4) 4 (20)	220 20	Other disease: No Yes
0.109	2.5	1	21 (10.6) 8 (19.5)	199 41	Other animal: No Yes
0.114	2.4	1	19 (10.3) 10 (18.2)	185 55	Season: Dry Wet
0.208	1.5	1	19 (10.6) 10 (16.7)	180 60	Type of soil: Mud Sand
0.114	2.4	1	19 (10.3) 10 (18.2)	185 55	Rainfall: Absent Present
0.263	1.2	1	6 (8.5) 23 (13.6)	71 169	Vegetation: Absent Poor
0.467	0.52	1	19 (11.1) 10 (14.5)	171 69	Temperature(c <sup>0</sup> ): < 40 > 40
0.467	0.52	1	19 (11.1) 10 (14.5)	171 69	Humidity (%): < 40 > 40
0.360	3.2	3	3 (7.5) 1 (5.9)	40 17	Locality: Omdurman Khartoum

			4 (22.2)	18	Um bada
			21 (12.7)	165	East Nile

### Multivariate analysis using Logistic Regression models:

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

**Table 3.5:**

Final logistic regression model of sheep Haemonchosis in Khartoum state, Sudan (June, 2013 to August, 2013;  $n=240$ )

<i>p- value</i>	95% Confidence Interval for Exp.(B)		Exp(B)	Animals affected (%)	Risk factors
	Upper	Lower			
0.000			Ref	2 (3.7)	Age:
	22.20	1.35	2.82	11 (8.9)	<1 yr
	72.24	4.89	8.03	16 (25.8)	1-3 year > 3 Year
0.004			Ref	7 (8)	Breed:
	0.75	0.21	0.35	10 (9.5)	Hamari
	20.78	1.02	4.60	12 (26.7)	Ashgar Kabashi
0.000			Ref	12 (6.3)	Fecal consistency:
	121.39	5.30	25.2	16 (32)	Normal Soft

	550	4.63	159.6	2	(50)	Diarrhea
0.009	0.255	0.004	Ref 0.33	13 16	(8.2) (19.7)	Grazing: Semi nomadic Zero grazing
0.000	254	7.40	Ref 43.43	10 (6.5)19	(21.8)	Use of Anthelmintics: Yes No

## Chapter Four

### Discussion

Sheep are considered as one of the most important livestock providing meat, milk and wool. Sheep husbandry could be improved by eliminating the antagonistic factors. One of the most significant of these factors is parasitic diseases, especially those caused by nematodes, which can act as stressful factor that depresses the productivity of these animals. This loss of production is clearly associated with anemia, emaciation and marked retardation of growth, resulting in huge economic losses (El-dakhly *et al.*, 2012).

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 Sudan with it is estimated 40 million head of sheep, and is considered as one of the largest sheep breeding

countries in Africa. Therefore great attention should be directed towards ovine Haemonchosis in Sudan (Fayza *et al.*, 2003).

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 was 12.1%. 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).

The prevalence of sheep Haemonchosis in this 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) 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), in Pakistan 37.18 % and 35.44% (Qamer *et al.*, 2009; Raza *et al.*, 2009). However, the prevalence of sheep Haemonchosis in this study is much higher than the prevalence in Iran which was 9.3% (Tehrani *et al.*, 2012). The results of this study showed that the prevalence of Haemonchosis within different age group of sheep has a highly significant association (p-value = 0.000). Animals with more than 3 years of age were highly affected (25.8%), compared with animals from 1-3 years of age (8.9%) and animals less than 1 year of age (3.7%). The



difference in the Infection rate could be referred to the fact that aged animals have a longer exposure time to *Haemonchus contortus* in pastures; several studies have shown that mature animals can become refractory to further infection when subjected to prolonged and repeated challenge (Fayza et al., 2003). These results are in agreement with the Previous report of Tasawar in a study conducted in Punjab, Pakistan (Tasawar et al., 2010)also our study in agreement with study conducted in Behshahr Iran (Gerdaghiet al., 2013).This results do not correspond with the studies carried out in Pakistan (Lateef et al., 2006) and Kenya (Ng'an'ga et al., 2004). Our results not coincide with the studies carried out in Pakistan and in Ethiopia which did not show any significant difference (Qamer et al., 2009; Sissay, 2007; Nagasi et al.,2012 ;Dagnachew et al., 2011). The Prevalence of *Haemonchus contortus* infection related tobreed of animals was 26.7% in Kabashi Breed, 8% in Hamari breed and 9.5% in Ashgar breed. There was highly significant association between breed and *Haemonchus contortus* infection (p-value=0.004). Kabashi breed has a higher rate of infection. This could be attributed to the difference in mode of infective larvae acquisition and larval development into adult worm. In generally accepted that immune response plays a vital role in the demonstration of inheriting resistance. These findings are consistent with observations reported in different breeds of sheep in Pakistan and also consistent with observations reported in different sheep breed in Pakistan

(Chuadary *et al.*, 2007) and disagreed 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).

The prevalence of *Haemonchus contortus* infection in animals with different faecal consistency was: 50% in animal with diarrhea, 32% in animals with soft faeces, and 6.3% in animals with normal faeces. There was significant association between *Haemonchus contortus* infection and faecal consistency (p-value=0.000). The animals with diarrhea have the highest rates of infection than others. This could be attributed to the fact that diarrhea affects tolerance against infection, and decreases the animals immunity.

In this study, the grazing type was investigated. The prevalence of infection was high in stationary animals (Zero grazing) which 19.7% as compared with animals in semi-nomadic system which 8.2%. There was significant association between ovine Haemonchosis and grazing type (p-value=0.009) This may be due to the spreading of eggs in the farm from infected settled animals to the healthy animals by the defecation near to feeds or due to chronic infection which induced some adaptive resistance.

The prevalence of *Haemonchus contortus* infection in relation to using of anthelmintics was 6.5% in animals whose owners used anthelmintics , 21.8% in animals whose owners did not use anthelmintics, There was highly significant association between *Haemonchous contortus* infection and using anthelmintics (p-value=0.000). Animals whose owners did not use anthelmintics have a higher rate of infection. This could be attributed to the fact that using of anthelmintics would reduce parasite infection. This finding in agreement with study carried out in North Kordofan state (Mubarak, 2013).

prevalence of infection was found in animals having other diseases (20 %) and in animals not show other diseases (11.4%). The reason that presence of other diseases play a major role in infection, because diseased animals have a little tolerance; due to little of immunity; and therefore more susceptible to Infection. There was no significant association between *Haemonchus contortus* infection and presence of other diseases (P-value =0.257).

Infection was more prevalent in sheep raised with other animals (19.5 %) than animals raised without other animals (10.6%), may be other animals act in the spreading of the disease. There was no significant association between

*Haemonchus contortus* infection and presence of other animals (p-value=0.109).

This result showed that infection percent was varying in the seasons; during wet season (18.2 %) and in the dry season (10.3%). This variation may be due to present of optimal environmental conditions for the parasite surviving and development during the wet season. However, there was no significant association between *Haemonchus contortus* infection and season (p-value=0.114). This finding in contrast with finding of abattoir study carried out in Sudan (Fayzaet *al.*, 2003), and in Ethiopia (Sissay, 2007). But this result disagrees with other studies conducted in Pakistan (Qamer *et al.*, 2009), Iran (Tahrani *et al.*, 2012). This difference in our opinion, might probably may be due to seasonal variation, and also as a result of larval inhibition during the dry periods of the year.

The results of this study showed that the prevalence of sheep Haemonchosis in different soil types was 10.6 % in mud soil and 16.7% in sand soil. There was no significant association between the *Haemonchus contortus* infection and type of soil (P-value = 0.208).

This result showed that infection percent was vary in the present (18.2 %) and absent (10.3%) of rainfall. There was no significant association between

*Haemonchus contortus* infection and rainfall status (p-value=0.114). This finding is in contrast with finding of abattoir study carried out in Sudan (Fayza *et al.*, 2003), and in Ethiopia (Sissay, 2007). But do not coincide with other study carried out in Pakistan (Qamer *et al.*, 2009).

Eleven risk factors were found to be significantly (P-value  $\leq$  0.25) associated with sheep Haemonchosis in the univariate analysis (Table 3.4) entered to logistic regression; final model, out of these eleven risk factors, five factors were significantly (P-value  $\leq$  0.05) associated with sheep Haemonchosis in the multivariate analysis. These five risk factors included: age of sheep, breed of sheep, faecal consistency, grazing and use of anthelmintics.

### **Conclusion:**

Sheep Haemonchosis was wide spread in Khartoum state, Sudan; the overall prevalence was 12.1% . Animals more than three years of age were highly affected compared to young animals; prevalence of sheep Haemonchosis was higher in Kabashi breed followed by Hamari and then Ashgar breed.

The prevalence was higher in animals having diarrhea followed by animals having soft faecal consistency and then animals having normal faecal consistency.

The prevalence was higher in stationary animals which in semi nomadic grazing compared with animals in zero grazing. The infection rate of *Haemonchus contortus* was higher in animals which their owners did not use anthelmintics than animals which their owners used anthelmintics.

### **Recommendation:**

- Knowledge of the disease epidemiology in the county; which vary from place to another according to the agricultural and ecological variation.

- Awareness of animal owner's about the disease, treatment and the control strategy.
- Continuous extension about the disease treatment and control strategy.
  - Implementation of pasture control to reduce larval contamination and decrease animal density in pasture and at watering points particularly during dry season.
  - Prophylactic using of anthelmintics with the knowledge of other factors which associate with Haemonchosis.
  - The control of Haemonchosis must be done by the integration of several methods; because each method has it is own advantages and disadvantages.

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## **Appendix I**

### **Questionnaire:**



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

Date \_\_\_\_\_ Locality: \_\_\_\_\_

Address \_\_\_\_\_ Herd Code \_\_\_\_\_

**Individual risk factors;-**

I- Age ;( years)

<1 year (     ), 1-3years (     ), >3years(     ).

II- Sex;   male(     ),female (     ).

III- Breed;

    Hamari (     ), Kabashi (     ), Ashgur (     ),..... (     ).

IV- Body condition score;

Good (     ), moderate (     ), poor (     ).

V- Faecal consistency;

Normal (     ), soft (     ),diarrhea (     ).

VI - Source of animal addition to the herd;

Raised on farm (     ).

Purchased from other farm (     ).

Purchased from local market (     ).

**2-Managerial Factors;**

I- Herd size; - 1 to 35 ( ), 36 to 70( ), >70( ).

II- Grazing type;-

Nomadic ( ) Semi nomadic ( ), stationary (zero grazing) ( ).

III- Housing;

Indoor ( ), outdoor ( ).

IV- use anti helminthes;-

Yes ( ), No ( ).

V- Presence of other animal's spp; -

Yes ( ), No ( ).

VI- Other disease status;

Yes ( ), No ( ).

**3-Climatical risk factors;-**

I -Seasons:

Dry ( ), wet ( ).

II- Types of soil;-

Mud ( ) , sand ( ).

III – Rainfall:

Present ( ), absent ( ).

IV- Vegetation:

Good (    ),    poor (    ).

V- Temperature (°C):

< 40 (    ) , > 40 (    ).

VI -Humidity (%):

< 40 (    ) , > 40 (    ).

VII -Provinces (Localities):

Omdurman (    ), Khartoum (    ), Um Bada (    ) and East Nile (    ).

## Appendix II

3.2- Frequency table for the distribution of 240 sheep examined for Haemonchosis by epg count and faecal culture according to potential risk factors

**Table 3.2.1: Frequency distribution of age:**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	<1year	54	22.5	22.5	22.5
	1-3yr	124	51.7	51.7	74.2
	> 3 yrs	62	25.8	25.8	100
	Total	240	100	100	

**Table 3.2.2: Frequency distribution of sex**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	113	47.1	47.1	47.1
	Female	127	52.9	52.9	100
	Total	240	100	100	

**Table 3.2.3: Frequency distribution of breed:**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Ashgar	105	43.8	43.8	43.8
	Hamari	90	37.5	37.5	81.3
	Kabashi	45	18.8	18.8	100
	Total	240	100	100	

**Table 3.2.4: Frequency table of body condition:**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Good	150	62.5	62.5	62.5
	Moderate	88	36.7	36.7	99.2
	Poor	2	0.8	0.8	100
	Total	240	100	100	

**Table 3.2.5: Frequency distribution of faecal consistency**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Normal	188	78.3	78.3	78.3
	Soft	50	20.7	20.7	99.2
	Diarrhea	2	0.8	0.8	100
	Total	240	100	100	

**Table 3.2.6: Frequency distribution to source of animal additions**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	raised on farm	187	77.9	77.9	77.9
	Purchased from farm	28	11.7	11.7	89.6
	Purchased from local markets	25	10.4	10.4	100
	Total	240	100	100	

**Table 3.2.7: Frequency distribution of herd size**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1-35 animals	62	25.8	25.8	25.8
	36-70 animals	123	51.3	51.3	77.1
	>70 animals	55	22.9	22.9	100
	Total	240	99.6	100	

**Table 3.2.8: Frequency distribution of housing system**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Outdoor	147	61.3	61.3	61.3
	Indoor	93	38.7	38.7	100
	Total	240	100	100	

**Table 3.2.9: Frequency distribution of grazing system:**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Zero grazing	81	33.8	33.8	33.8
	Semi nomadic	159	66.3	66.3	100.0
	Total	240	100	100	

**Table 3.2.10: Frequency distribution of using anthelmintics**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	153	63.7	63.7	63.7
	No	87	36.3	36.3	100.0
	Total	240	100	100	

**Table 3.2.11: Frequency distribution of other disease status**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	220	91.7	91.7	91.7
	Yes	20	8.3	8.3	100
	Total	240	100	100	



**Table 3.2.12: Frequency distribution of other animalsmixing**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	199	82.9	82.9	82.9
	Yes	41	17.1	17.1	100
	Total	240	100	100	

**Table 3.2.13: Frequency distribution of season**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Dry	185	77.1	77.1	77.1
	Wet	55	22.9	22.9	100
	Total	240	100	100	

**Table 3.2.14: Frequency distribution of soil type**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Mud	180	75.0	75.0	75.0
	Sand	60	25.0	25.0	100
	Total	240	100	100	

**Table 3.2.15: Frequency distribution of rainfall status**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	185	77.1	77.1	77.1
	Present	55	22.9	22.9	100
	Total	240	100	100	

**Table 3.2.16: Frequency distribution of vegetation status**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	71	29.6	29.6	29.6
	Poor	169	70.4	70.4	100
	Total	240	100	100	

**Table 3.2.17: Frequency distribution of temperature (c<sup>o</sup>)**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	<40	171	71.3	71.3	71.3
	> 40	69	28.7	28.7	100
	Total	240	100	100	

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**Table 3.2.18: Frequency distribution of humidity (%)**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	< 40	171	71.3	71.3	71.3
	>40	69	28.7	28.7	100
	Total	240	100	100	

**Table 3.2.19: Frequency distribution of animal tested in the localities**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Omdurman	40	16.7	16.7	16.7
	Khartoum	17	7.1	7.1	23.8
	Um bada	18	7.5	7.5	31.3
	East Nile	165	68.8	68.8	100
	Total	240	100	100	

### Appendix III

**3.3 Cross-tabulation:** Cross-tabulation of sheep Haemonchosis examined by the egg per gram (epg) count and faecal culture with potential risk factors investigated.

Table 3.3.1: sheep Haemonchosis and age cross-tabulation

Count		Age			Total
		less than 1year	1-3yr	more than 3yr	
Outcome	-ve	52 (52/54x100 =96.3) (%)	113 (113/124x100 =91.1)	46 (46/62x100 =74.2)	211 (211/240x100 =87.9)
	+ve	2 (2/54x100 =3.7) (%)	11 (11/124x100 =8.9)	16 (16/62x100 =25.8)	29 (29/240x100 =12.1)
Total		54	124	62	240

Table 3.3.2: sheep Haemonchosis and sheep sex cross-tabulation

Count		Sex		Total
		Male	Female	
Outcome	-ve	102 (90.3) (%)	109 (85.8)	211 (87.9)
	+ve	11 (9.7) (%)	18 (14.2)	29 (12.1)

Total	113	127	240
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Table 3.3.3: sheep Haemonchosis and breed cross-tabulation

Count		Breed			Total
		Ashgar	Hamari	Kabashi	
Outcome	-ve (%)	95 (90.5)	83 (92)	45 (88)	211 (87.9)
	+ve (%)	10 (9.5)	7 (8)	12 (26.7)	29 (12.1)
Total					240

Table 3.3.4: sheep Haemonchosis and body condition cross-tabulation

Count		Body condition			Total
		Good	Moderate	Poor	
Outcome	-ve (%)	133 (88.7)	77 (87.5)	1 (50)	211 (87.9)
	+ve (%)	17 (11.3)	11 (12.5)	1 (50)	29 (12.1)
Total		150	88	2	240

Table 3.3.5: sheep Haemonchosis and faecal consistency cross-tabulation

Count		Faecal consistency			Total
		Normal	Soft	Diarrhea	
Outcome	-ve (%)	176 (93.6)	34 (68)	1 (50)	211 (87.9)
	+ve (%)				
Total					

	+ve	12	16	1	29
	(%)	(6.4)	(32)	(50)	(12.1)
Total		188	50	2	240

Table 3.3.6: sheep Haemonchosis and source of animal cross-tabulation

Count	Source of animal			Total
	rasied on farm	purchased from farm	from local markets	
Outcome -ve	167	24	20	211
(%)	(89.3)	(85.7)	(80)	(87.9)
+ve	20	4	5	29
(%)	(10.7)	(14.3)	(20)	(12.1)
Total	187	28	25	240

Table 3.3.7: sheep Haemonchosis and herd size cross-tabulation

Count	Herd size			Total
	1-35	36-70	more than 70	
Outcome -ve	53	108	50	211
(%)	(85.5)	(87.8)	(90.9)	(87.9)
+ve	9	15	5	29
(%)	(14.5)	(12.2)	(9.1)	(12.1)
Total	62	123	55	240

Table 3.3.8: sheep Haemonchosis and housing cross-tabulation

Count	Housing		Total
	Outdoor	Indoor	
Outcome			
-ve (%)	130 (88.4)	81 (87.1)	211 (87.9)
+ve (%)	17 (11.6)	12 (12.9)	29 (12.1)
Total	147	93	240

Table 3.3.9: sheep Haemonchosis and grazing cross-tabulation

Count	Grazing		Total
	Zero grazing	Semi- nomadic	
Outcome			
-ve (%)	65 (80.3)	146 (92.8))	211 (87.9)
+ve (%)	16 (19.7)	13 (8.2)	29 (12.1)
Total	81	159	240

Table 3.3.10: sheep Haemonchosis and use of anthelmintics cross-tabulation

Count		Use of Anthelmintics		Total
		Use	not use	
Outcome	-ve (%)	143 (93.5)	68 (78.2)	211 (87.9)
	+ve (%)	10 (6.5)	19 (21.8)	29 (12.1)
Total		153	87	240

Table 3.3.11: sheep Haemonchosis and other disease status cross-tabulation

Count		Other disease		Total
		No	Yes	
Outcome	-ve (%)	195 (88.6)	16 (80)	211 (8.9)
	+ve (%)	25 (11.4)	4 (20)	29 (12.1)
Total		220	20	240

Table 3.3.12: sheep Haemonchosis and presence of other animals spp cross-tabulation

Count		Other animals		Total
		No	Yes	
Outcome	-ve (%)	178 (89.4)	33 (80.5)	211 (87.9)
	+ve (%)	21 (10.6)	8 (19.5)	29 (12.1)
Total		199	41	240

Table 3.3.13: sheep Haemonchosis and season cross-tabulation



Count	Season		Total
	Dry	Wet	
Outcome -ve (%)	166 (89.7)	45 (81.8)	211 (87.9)
+ve (%)	19 (10.3)	10 (18.2)	29 (12.1)
Total	185	55	240

Table 3.3.14: sheep Haemonchosis and type of soil cross-tabulation

Count	Type of soil		Total
	Mud	Sand	
Outcome -ve (%)	161 (89.4)	50 (83.3)	211 (87.9)
+ve (%)	19 (10.6)	10 (16.7)	29 (12.1)
Total	180	60	240

Table 3.3.15: sheep Haemonchosis and rainfall cross-tabulation

Count	Rainfall		Total
	Absent	Present	
Outcome -ve (%)	166 (89.7)	45 (81.8)	211 (87.9)
+ve (%)	19 (10.3)	10 (18.2)	29 (12.1)
Total	185	55	240

Table 3.3.16: sheep Haemonchosis and vegetation cross-tabulation

Count	Vegetation		Total
	Absent	Poor	
Outcome -ve (%)	65 (91.5)	146 (86.4)	211 (87.9)
+ve (%)	6 (8.5)	23 (13.6)	29 (12.1)
Total	71	169	240

Table 3.3.17: sheep Haemonchosis and temperature cross-tabulation

Count	Temperature (c <sup>0</sup> )		Total
	Less than 40	more than40	
Outcome -ve (%)	152 (88.9)	59 (85.5)	211 (87.9)
+ve (%)	19 (11.1)	10 (14.5)	29 (12.1)
Total	171	69	240

Table 3.3.18: sheep Haemonchosis and humidity cross-tabulation

Count	Humidity(%)		Total
	Less than 30	More than 30	
Outcome -ve (%)	152 (88.9)	59 (85.5)	211 (87.9)
+ve (%)	19 (11.1)	10 (14.5)	29 (12.1)
Total	171	69	240

Table 3.3.19: sheep Haemonchosis and locality cross-tabulation

Count	Locality				Total
	Omdurman	Khartoum	Um bada	East Nile	
Outcome -ve (%)	37 (92.5)	16 (94.1)	14 (77.8)	144 (87.3)	211 (87.9)
+ve (%)	3 (7.5)	1 (5.9)	4 (22.2)	21 (12.7)	29 (12.1)
Total	40	17	18	165	240

## Appendix IV

3.4 Univariate analysis for the association of sheep Haemonchosis in 240 sheep examined by egg per gram (epg) count and faecal culture with potential risk factors using Chi square ( $\chi^2$ ) test.

Table 3.4.1: Age

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	15.765	2	.000
Likelihood Ratio	14.718	2	.001
Linear-by-Linear Association	13.759	1	.000
N of Valid Cases	240		

Table 3.4.2: Sex

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.109	1	.292
Likelihood Ratio	1.122	1	.290
Linear-by-Linear Association	1.104	1	.293
N of Valid Cases	240		

Table 3.4.3: Breed

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	11.227	2	.004
Likelihood Ratio	9.488	2	.009
Linear-by-Linear Association	5.941	1	.015
N of Valid Cases	240		

Table 3.4.4: Body condition

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.800	2	.247
Likelihood Ratio	1.807	2	.405
Linear-by-Linear Association	.547	1	.459
N of Valid Cases	240		

Table 3.4.5: Fecal consistency

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	27.127	2	.000
Likelihood Ratio	22.206	2	.000
Linear-by-Linear Association	26.921	1	.000
N of Valid Cases	240		

Table 3.4.6: Source of animal

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.942	2	.399
Likelihood Ratio	1.737	2	.420
Linear-by-Linear Association	1.911	1	.167
N of Valid Cases	240		

Table 3.4.7: Herd Size

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.810	2	.667
Likelihood Ratio	.830	2	.660
Linear-by-Linear Association	.798	1	.372
N of Valid Cases	240		

Table 3.4.8: Housing

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.096	1	.757
Likelihood Ratio	.011	1	.915
Linear-by-Linear Association	.095	1	.757
N of Valid Cases	240		

Table: 3.4.9: Grazing

	Value	df	Asymp. Sig. (2-sided)
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Pearson Chi-Square	6.770	1	.009
Likelihood Ratio	5.724	1	.017
Linear-by-Linear Association	6.402	1	.011
N of Valid Cases	240		

Table 3.4.10: Use of anthelmintics:

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	12.227	1	.000
Likelihood Ratio	10.828	1	.001
Linear-by-Linear Association	11.704	1	.001
N of Valid Cases	240		

Table 3.4.11: Other disease

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.287	1	.257
Likelihood Ratio	0.603	1	.438
Fisher's Exact Test			
Linear-by-Linear Association	1.121	1	.290
N of Valid Cases	240		

Table 3.4.12: Other animals

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.569	1	.109
Likelihood Ratio	1.795	1	.180
Linear-by-Linear Association	2.297	1	.130
N of Valid Cases	240		

Table 3.4.13: Season

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.498	1	.114
Likelihood Ratio	1.809	1	.179
Linear-by-Linear Association	2.301	1	.129
N of Valid Cases	240		

Table 3.4.14: Type of soil:

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.582	1	.208
Likelihood Ratio	1.059	1	.303
Linear-by-Linear Association	1.488	1	.222
N of Valid Cases	240		

Table 3.4.15: Rainfall

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.498	1	.114
Likelihood Ratio	1.809	1	.179
Linear-by-Linear Association	2.301	1	.129
N of Valid Cases	240		

Table 3.4.16: Vegetation

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.252	1	.263
Likelihood Ratio	0.814	1	.367
Linear-by-Linear Association	1.330	1	.249
N of Valid Cases	240		

Table 3.4.17: Temperature (c<sup>0</sup>)

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.529	1	.467
Likelihood Ratio	.259	1	.611
Linear-by-Linear Association	.513	1	.474
N of Valid Cases	240		

Table 3.4.18: Humidity (%)

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.529	1	.467



Likelihood Ratio	.259	1	.611
Fisher's Exact Test			
Linear-by-Linear Association	.513	1	.474
N of Valid Cases	240		

Table 3.4.19: Locality

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.213	3	.360
Likelihood Ratio	3.147	3	.370
Linear-by-Linear Association	.971	1	.324
N of Valid Cases	240		